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(54) **Combinations of hepatitis C virus (HCV) antigens for use in immunoassays for anti-HCV antibodies.**

(57) **Combinations of HCV antigens that have a broader range of immunological reactivity than any single HCV antigen. The combinations consist of an antigen from the C domain of the HCV polypeptide, and at least one additional HCV antigen from either the NS3 domain, the NS4 domain, the S domain, or the NS5 domain, and are in the form of a fusion protein, a simple physical mixture, or the individual antigens commonly bound to a solid matrix.**

**EP 0 450 931 A1**

Technical Field

The present invention is in the field of immunoassays for HCV (previously called Non-A, Non-B hepatitis virus). More particularly, it concerns combinations of HCV antigens that permit broad range immunoassays for anti-HCV antibodies.

Background

The disease known previously as Non-A, Non-B hepatitis (NANBH) was considered to be a transmissible disease or family of diseases that were believed to be viral-induced, and that were distinguishable from other forms of viral-associated liver diseases, including that caused by the known hepatitis viruses, i.e., hepatitis A virus (HAV), hepatitis B virus (HBV), and delta hepatitis virus (HDV), as well as the hepatitis induced by cytomegalovirus (CMV) or Epstein-Barr virus (EBV). NANBH was first identified in transfused individuals. Transmission from man to chimpanzee and serial passage in chimpanzees provided evidence that NANBH was due to a transmissible infectious agent or agents. Epidemiologic evidence suggested that there may be three types of NANBH: a water-borne epidemic type; a blood-borne or parenterally transmitted type; and a sporadically occurring (community acquired) type. However, until recently, no transmissible agent responsible for NANBH had been identified, and clinical diagnosis and identification of NANBH had been accomplished primarily by exclusion of other viral markers. Among the methods used to detect putative NANBH antigens and antibodies were agar-gel diffusion, counterimmunoelectrophoresis, immunofluorescence microscopy, immune electron microscopy, radioimmunoassay, and enzyme-linked immunosorbent assay. However, none of these assays proved to be sufficiently sensitive, specific, and reproducible to be used as a diagnostic test for NANBH.

In 1987, scientists at Chiron Corporation (the owner of the present application) identified the first nucleic acid definitively linked to blood-borne NANBH. See, e.g., EPO Pub. No. 318,216; Houghton et al., *Science* 244:359 (1989). These publications describe the cloning of an isolate from a new viral class, hepatitis C virus (HCV), the prototype isolate described therein being named "HCV1." HCV is a Flavi-like virus, with an RNA genome.

U.S. Patent Application Serial No. 456,637 (Houghton et al.), incorporated herein by reference, describes the preparation of various recombinant HCV polypeptides by expressing HCV cDNA and the screening of those polypeptides for immunological reactivity with sera from HCV patients. That limited screening showed that at least five of the polypeptides tested were very immunogenic; specifically, those identified as 5-1-1, C100, C33c, CA279a, and CA290a. Of these five polypeptides, 5-1-1 is located in the putative NS4 domain; C100 spans the putative NS3 and NS4 domains; C33c is located within the putative NS3 domain and CA279a and CA290a are located within the putative C domain. The screening also showed that no single polypeptide tested was immunologically reactive with all sera. Thus, improved tests, which react with all or more samples from HCV positive individuals, are desirable.

Disclosure of the Invention

Applicants have carried out additional serological studies on HCV antigens that confirm that no single HCV polypeptide identified to date is immunologically reactive with all sera. This lack of a single polypeptide that is universally reactive with all sera from individuals with HCV may be due, *inter alia*, to strain-to-strain variation in HCV epitopes, variability in the humoral response from individual-to-individual and/or variation in serology with the state of the disease.

These additional studies have also enabled applicants to identify combinations of HCV antigens that provide more efficient detection of HCV antibodies than any single HCV polypeptide.

Accordingly, one aspect of this invention is a combination of HCV antigens comprising:

- (a) a first HCV antigen from the C domain; and
- (b) at least one additional HCV antigen selected from the group consisting of
  - (i) an HCV antigen from the NS3 domain;
  - (ii) an HCV antigen from the NS4 domain;
  - (iii) an HCV antigen from the S domain; and
  - (iv) an HCV antigen from the NS5 domain.

In one embodiment, the combination of HCV antigens is in the form of a fusion protein comprised of the antigens. In an alternative embodiment, the combination of antigens is in the form of the individual antigens bound to a common solid matrix. In still another embodiment, the combination of antigens is in the form of a mixture of the individual antigens.

Another aspect of the invention is a method for detecting antibodies to HCV in a mammalian body compo-

nent suspected of containing said antibodies comprising contacting said body component with the above-described combination of HCV antigens under conditions that permit antibody-antigen reaction and detecting the presence of immune complexes of said antibodies and said antigens.

Another aspect of the invention is a method for detecting antibodies to HCV in a mammalian body component suspected of containing said antibodies comprising contacting said body component with a panel of HCV antigens, simultaneously or sequentially, comprising

- (a) a first HCV antigen from the C domain; and
- (b) at least one additional HCV antigen selected from the group consisting of
  - (i) an HCV antigen from the NS3 domain;
  - (ii) an HCV antigen from the NS4 domain;
  - (iii) an HCV antigen from the S domain; and
  - (iv) an HCV antigen from the NS5 domain under conditions that permit antibody-antigen reaction and detecting the presence of immune complexes of said antibodies and said antigens.

Another aspect of the invention is a kit for carrying out an assay for detecting antibodies to HCV in a mammalian body component suspected of containing said antibodies comprising in packaged combination

- (a) said combination of HCV antigens;
- (b) standard control reagents; and
- (c) instructions for carrying out the assay.

#### Brief Description of the Drawings

In the drawings:

Figure 1 is the nucleotide sequence of the cDNA sense and anti-sense strand for the HCV polyprotein and the amino acid sequence encoded by the sense strand.

Figure 2 is a schematic of the amino acid sequence of Figure 1 showing the putative domains of the HCV polypeptide.

#### Modes for Carrying Out the Invention

#### Definitions

"HCV antigen" intends a polypeptide of at least about 5 amino acids, more usually at least about 8 to 10 amino acids that defines an epitope found in an isolate of HCV. Preferably, the epitope is unique to HCV. When an antigen is designated by an alphanumeric code, the epitope is from the HCV domain specified by the alphanumeric.

"Synthetic" as used to characterize an HCV antigen intends that the HCV antigen has either been isolated from native sources or man-made such as by chemical or recombinant synthesis.

"Domains" intends those segments of the HCV polyprotein shown in Figure 2 which generally correspond to the putative structural and nonstructural proteins of HCV. Domain designations generally follow the convention used to name Flaviviral proteins. The locations of the domains shown in Figure 2 are only approximate. The designations "NS" denotes "nonstructural" domains, while "S" denotes the envelope domain, and "C" denotes the nucleocapsid or core domain.

"Fusion polypeptide" intends a polypeptide in which the HCV antigen(s) are part of a single continuous chain of amino acids, which chain does not occur in nature. The HCV antigens may be connected directly to each other by peptide bonds or be separated by intervening amino acid sequences. The fusion polypeptides may also contain amino acid sequences exogenous to HCV.

"Common solid matrix" intends a solid body to which the individual HCV antigens or the fusion polypeptide comprised of HCV antigens are bound covalently or by noncovalent means such as hydrophobic adsorption.

"Mammalian body component" intends a fluid or tissue of a mammalian individual (e.g., a human) that commonly contains antibodies produced by the individual. Such components are known in the art and include, without limitation, blood, plasma, serum, spinal fluid, lymph fluid, secretions of the respiratory, intestinal or genitourinary tracts, tears, saliva, milk, white blood cells, and myelomas.

"Immunologically reactive" means that the antigen in question will react specifically with anti-HCV antibody commonly present in a significant proportion of sera from individuals infected with HCV.

"Immune complex" intends the combination or aggregate formed when an antibody binds to an epitope on an antigen.

### Combinations of HCV Antigens

Figure 2 shows the putative domains of the HCV polyprotein. The domains from which the antigens used in the combinations derive are: C, S (or E), NS3, NS4, and NS5. The C domain is believed to define the nucleocapsid protein of HCV. It extends from the N-terminal of the polyprotein to approximately amino acid 120 of Figure 1. The S domain is believed to define the virion envelope protein, and possibly the matrix (M) protein, and is believed to extend from approximately amino acid 120 to amino acid 400 of Figure 1. The NS3 domain extends from approximately amino acid 1050 to amino acid 1640 and is believed to constitute the viral protease. The NS4 domain extends from the terminus of NS3 to approximately amino acid 2000. The function of the NS4 protein is not known at this time. Finally, the NS5 domain extends from about amino acid 2000 to the end of the polyprotein and is believed to define the viral polymerase.

The sequence shown in Figure 1 is the sequence of the HCV1 isolate. It is expected that the sequences of other strains of the blood-borne HCV may differ from the sequence of Figure 1, particularly in the envelope (S) and nucleocapsid (C) domains. The use of HCV antigens having such differing sequences is intended to be within the scope of the present invention, provided, however, that the variation does not significantly degrade the immunological reactivity of the antigen to sera from persons infected with HCV.

In general, the HCV antigens will comprise entire or truncated domains, the domain fragments being readily screened for antigenicity by those skilled in the art. The individual HCV antigens used in the combination will preferably comprise the immunodominant portion (i.e., the portion primarily responsible for the immunological reactivity of the polypeptide) of the stated domain. In the case of the C domain it is preferred that the C domain antigen comprise a majority of the entire sequence of the domain. The antigen designated C22 (see Example 4, *infra*), is particularly preferred. The S domain antigen preferably includes the hydrophobic subdomain at the N-terminal end of the domain. This hydrophobic subdomain extends from approximately amino acid 199 to amino acid 328 of Figure 1. The HCV antigen designated S2 (see Example 3, *infra*), is particularly preferred. Sequence downstream of the hydrophobic subdomain may be included in the S domain antigen if desired.

A preferred NS3 domain antigen is the antigen designated C33c. That antigen includes amino acids 1192 to 1457 of Figure 1. A preferred NS4 antigen is C100 which comprises amino acids 1569 to 1931 of Figure 1. A preferred NS5 antigen comprises amino acids 2054 to 2464 of Figure 1.

The HCV antigen may be in the form of a polypeptide composed entirely of HCV amino acid sequence or it may contain sequence exogenous to HCV (i.e., it may be in the form of a fusion protein that includes exogenous sequence). In the case of recombinantly produced HCV antigen, producing the antigen as a fusion protein such as with SOD, alpha-factor or ubiquitin (see commonly owned U.S. Pat. No. 4,751,180, U.S. Pat. No. 4,870,008 and U.S. Pat. Application Serial. No. 390,599, filed 7 August 1989, the disclosures of which are incorporated herein, which describe expression of SOD, alpha-factor and ubiquitin fusion proteins) may increase the level of expression and/or increase the water solubility of the antigen. Fusion proteins such as the alpha-factor and ubiquitin fusion are processed by the expression host to remove the heterologous sequence. Alpha-factor is a secretion system, however, while ubiquitin fusions remain in the cytoplasm.

Further, the combination of antigens may be produced as a fusion protein. For instance, a continuous fragment of DNA encoding C22 and C33c may be constructed, cloned into an expression vector and used to express a fusion protein of C22 and C33c. In a similar manner fusion proteins of C22 and C100; C22 and S2; C22 and an NS5 antigen; C22, C33c, and S2; C22, C100 and S2, and C22, C33c, C100, and S2 may be made. Alternative fragments from the exemplified domain may also be used.

### Preparation of HCV Antigens

The HCV antigens of the invention are preferably produced recombinantly or by known solid phase chemical synthesis. They may, however, also be isolated from dissociated HCV or HCV particles using affinity chromatography techniques employing antibodies to the antigens.

When produced by recombinant techniques, standard procedures for constructing DNA encoding the antigen, cloning that DNA into expression vectors, transforming host cells such as bacteria, yeast, insect, or mammalian cells, and expressing such DNA to produce the antigen may be employed. As indicated previously, it may be desirable to express the antigen as a fusion protein to enhance expression, facilitate purification, or enhance solubility. Examples of specific procedures for producing representative HCV antigens are described in the Examples, *infra*, and in parent application Serial No. 456,637.

### Formulation of Antigens for Use in Immunoassay

The HCV antigens may be combined by producing them in the form of a fusion protein composed of two

or more of the antigens, by immobilizing them individually on a common solid matrix, or by physically mixing them. Fusion proteins of the antigen may also be immobilized on (bound to) a solid matrix. Methods and means for covalently or noncovalently binding proteins to solid matrices are known in the art. The nature of the solid surface will vary depending upon the assay format. For assays carried out in microtiter wells, the solid surface will be the wall of the well or cup. For assays using beads, the solid surface will be the surface of the bead. In assays using a dipstick (i.e., a solid body made from a porous or fibrous material such as fabric or paper) the surface will be the surface of the material from which the dipstick is made. In agglutination assays the solid surface may be the surface of latex or gelatin particles. When individual antigens are bound to the matrix they may be distributed homogeneously on the surface or distributed thereon in a pattern, such as bands so that a pattern of antigen binding may be discerned.

Simple mixtures of the antigens comprise the antigens in any suitable solvent or dispersing medium.

#### Assay Formats Using Combinations of Antigens

The HCV antigens may be employed in virtually any assay format that employs a known antigen to detect antibodies. A common feature of all of these assays is that the antigen is contacted with the body component suspected of containing HCV antibodies under conditions that permit the antigen to bind to any such antibody present in the component. Such conditions will typically be physiologic temperature, pH and ionic strength using an excess of antigen. The incubation of the antigen with the specimen is followed by detection of immune complexes comprised of the antigen.

Design of the immunoassays is subject to a great deal of variation, and many formats are known in the art. Protocols may, for example, use solid supports, or immunoprecipitation. Most assays involve the use of labeled antibody or polypeptide; the labels may be, for example, enzymatic, fluorescent, chemiluminescent, radio-active, or dye molecules. Assays which amplify the signals from the immune complex are also known; examples of which are assays which utilize biotin and avidin, and enzyme-labeled and mediated immunoassays, such as ELISA assays.

The immunoassay may be, without limitation, in a heterogeneous or in a homogeneous format, and of a standard or competitive type. In a heterogeneous format, the polypeptide is typically bound to a solid matrix or support to facilitate separation of the sample from the polypeptide after incubation. Examples of solid supports that can be used are nitrocellulose (e.g., in membrane or microtiter well form), polyvinyl chloride (e.g., in sheets or microtiter wells), polystyrene latex (e.g., in beads or microtiter plates, polyvinylidene fluoride (known as Immulon™), diazotized paper, nylon membranes, activated beads, and Protein A beads. For example, Dynatech Immulon™ 1 or Immulon™ 2 microtiter plates or 0.25 inch polystyrene beads (Precision Plastic Ball) can be used in the heterogeneous format. The solid support containing the antigenic polypeptides is typically washed after separating it from the test sample, and prior to detection of bound antibodies. Both standard and competitive formats are known in the art.

In a homogeneous format, the test sample is incubated with the combination of antigens in solution. For example, it may be under conditions that will precipitate any antigen-antibody complexes which are formed. Both standard and competitive formats for these assays are known in the art.

In a standard format, the amount of HCV antibodies forming the antibody-antigen complex is directly monitored. This may be accomplished by determining whether labeled anti-xenogenic (e.g., anti-human) antibodies which recognize an epitope on anti-HCV antibodies will bind due to complex formation. In a competitive format, the amount of HCV antibodies in the sample is deduced by monitoring the competitive effect on the binding of a known amount of labeled antibody (or other competing ligand) in the complex.

Complexes formed comprising anti-HCV antibody (or, in the case of competitive assays, the amount of competing antibody) are detected by any of a number of known techniques, depending on the format. For example, unlabeled HCV antibodies in the complex may be detected using a conjugate of anti-xenogenic Ig complexed with a label, (e.g., an enzyme label).

In an immunoprecipitation or agglutination assay format the reaction between the HCV antigens and the antibody forms a network that precipitates from the solution or suspension and forms a visible layer or film of precipitate. If no anti-HCV antibody is present in the test specimen, no visible precipitate is formed.

The HCV antigens will typically be packaged in the form of a kit for use in these immunoassays. The kit will normally contain in separate containers the combination of antigens (either already bound to a solid matrix or separate with reagents for binding them to the matrix), control antibody formulations (positive and/or negative), labeled antibody when the assay format requires same and signal generating reagents (e.g., enzyme substrate) if the label does not generate a signal directly. Instructions (e.g., written, tape, VCR, CD-ROM, etc.) for carrying out the assay usually will be included in the kit.

The following examples are intended to illustrate the invention and are not intended to limit the invention

in any manner.

#### Example 1: Synthesis of HCV Antigen C33c

HCV antigen C33c contains a sequence from the NS3 domain. Specifically, it includes amino acids 1192-1457 of Figure 1. This antigen was produced in bacteria as a fusion protein with human superoxide dismutase (SOD) as follows. The vector pSODcf1 (Steiner et al. (1986), J. Virol. 58:9) was digested to completion with EcoRI and BamHI and the resulting EcoRI, BamHI fragment was ligated to the following linker to form pcf1EF:

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GATC CTG GAA TTC TGA TAA
GAC CTT AAG ACT ATT TTA A
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A cDNA clone encoding amino acids 1192-1457 and having EcoRI ends was inserted into pcf1EF to form pcf1EF/C33c. This expression construct was transformed into D1210 *E. coli* cells.

The transformants were used to express a fusion protein comprised of SOD at the N-terminus and in-frame C33c HCV antigen at the C-terminus. Expression was accomplished by inoculating 1500 ml of Luria broth containing ampicillin (100 micrograms/ml) with 15 ml of an overnight culture of the transformants. The cells were grown to an O.D. of 0.3, IPTG was added to yield a final concentration of 2 mM, and growth continued until the cells attained a density of 1 O.D., at which time they were harvested by centrifugation at 3,000 x g at 4°C for 20 minutes. The packed cells can be stored at -80°C for several months.

In order to purify the SOD-C33c polypeptide the bacterial cells in which the polypeptide was expressed were subjected to osmotic shock and mechanical disruption, the insoluble fraction containing SOD-C33c was isolated and subjected to differential extraction with an alkaline-NaCl solution, and the fusion polypeptide in the extract purified by chromatography on columns of S-Sepharose and Q-Sepharose.

The crude extract resulting from osmotic shock and mechanical disruption was prepared by the following procedure. One gram of the packed cells were suspended in 10 ml of a solution containing 0.02 M Tris HCl, pH 7.5, 10 mM EDTA, 20% sucrose, and incubated for 10 minutes on ice. The cells were then pelleted by centrifugation at 4,000 x g for 15 min at 4°C. After the supernatant was removed, the cell pellets were resuspended in 10 ml of Buffer A1 (0.01M Tris HCl, pH 7.5, 1 mM EDTA, 14 mM beta-mercaptoethanol [BME]), and incubated on ice for 10 minutes. The cells were again pelleted at 4,000 x g for 15 minutes at 4°C. After removal of the clear supernatant (periplasmic fraction I), the cell pellets were resuspended in Buffer A1, incubated on ice for 10 minutes, and again centrifuged at 4,000 x g for 15 minutes at 4°C. The clear supernatant (periplasmic fraction II) was removed, and the cell pellet resuspended in 5 ml of Buffer A2 (0.02 M Tris HCl, pH 7.5, 14 mM BME, 1 mM EDTA, 1 mM PMSF). In order to disrupt the cells, the suspension (5 ml) and 7.5 ml of Dyno-mill lead-free acid washed glass beads (0.10-0.15 mm diameter) (obtained from Glen-Mills, Inc.) were placed in a Falcon tube, and vortexed at top speed for two minutes, followed by cooling for at least 2 min on ice; the vortexing-cooling procedure was repeated another four times. After vortexing, the slurry was filtered through a scintered glass funnel using low suction; the glass beads were washed two times with Buffer A2, and the filtrate and washes combined.

The insoluble fraction of the crude extract was collected by centrifugation at 20,000 x g for 15 min at 4°C, washed twice with 10 ml Buffer A2, and resuspended in 5 ml of MILLI-Q water.

A fraction containing SOD-C33c was isolated from the insoluble material by adding to the suspension NaOH (2 M) and NaCl (2 M) to yield a final concentration of 20 mM each, vortexing the mixture for 1 minute, centrifuging at 20,000 x g for 20 min at 4°C, and retaining the supernatant.

In order to purify SOD-C33c on S-Sepharose, the supernatant fraction was adjusted to a final concentration of 6M urea, 0.05M Tris HCl, pH 7.5, 14 mM BME, 1 mM EDTA. This fraction was then applied to a column of S-Sepharose Fast Flow (1.5 x 10 cm) which had been equilibrated with Buffer B (0.05M Tris HCl, pH 7.5, 14 mM BME, 1 mM EDTA). After application, the column was washed with two column volumes of Buffer B. The flow through and wash fractions were collected. The flow rate of application and wash, was 1 ml/min; and collected fractions were 1 ml. In order to identify fractions containing SOD-C33c, aliquots of the fractions were analyzed by electrophoresis on 10% polyacrylamide gels containing SDS followed by staining with Coomassie blue. The fractions are also analyzable by Western blots using an antibody directed against SOD. Fractions containing SOD-C33c were pooled.

Further purification of SOD-C33c was on a Q-Sepharose column (1.5 x 5 cm) which was equilibrated with Buffer B. The pooled fractions containing SOD-C33c obtained from chromatography on S-Sepharose was applied to the column. The column was then washed with Buffer B, and eluted with 60 ml of a gradient of 0.0 to 0.4 M NaCl in Buffer B. The flow rate for application, wash, and elution was 1 ml/min; collected fractions

were 1 ml. All fractions from the Q-Sepharose column were analyzed as described for the S-Sepharose column. The peak of SOD-C33c eluted from the column at about 0.2 M NaCl.

The SOD-C33c obtained from the Q-Sepharose column was greater than about 90% pure, as judged by analysis on the polyacrylamide SDS gels and immunoblot using a monoclonal antibody directed against human SOD.

#### Example 2: Synthesis of HCV Antigen C100

HCV antigen C100 contains sequences from the NS3 and NS4 domains. Specifically, it includes amino acids 1569-1931 of Figure 1. This antigen was produced in yeast. A cDNA fragment of a 1270 bp encoding the above amino acids and heaving EcoRI termini was prepared.

The construction of a yeast expression vector in which this fragment was fused directly to the *S. cerevisiae* ADH2/GAP promoter was accomplished by a protocol which included amplification of the C100 sequence using a PCR method, followed by ligation of the amplified sequence into a cloning vector. After cloning, the C100 sequence was excised, and with a sequence which contained the ADH2/GAP promoter, was ligated to a large fragment of a yeast vector to yield a yeast expression vector.

The PCR amplification of C100 was performed using as template the vector pS3-56<sub>C100m</sub>, which had been linearized by digestion with Sall. pS3-56, which is a pBR322 derivative, contains an expression cassette which is comprised of the ADH2/GAPDH hybrid yeast promoter upstream of the human superoxide dismutase gene, and a downstream alpha factor transcription terminator.

The oligonucleotide primers used for the amplification were designed to facilitate cloning into the expression vector, and to introduce a translation termination codon. Specifically, novel 5'-HindIII and 3'-Sall sites were generated with the PCR oligonucleotides. The oligonucleotide containing the Sall site also encodes the double termination codons, TAA and TGA. The oligonucleotide containing the HindIII site also contains an untranslated leader sequence derived from the pgap63 gene, situated immediately upstream of the AUG codon. The pEco63GAPDH gene is described by Holland and Holland (1980) and by Kniskern et al. (1986). The PCR primer sequences used for the direct expression of C100m were:

5' GAG TGC TCA AGC TTC AAA ACA AAA TGG CTC  
ACT TTC TAT CCC AGA CAA AGC AGA GT 3'

and

5' GAG TGC TCG TCG ACT CAT TAG GGG GAA  
ACA TGG TTC CCC CGG GAG GCG AA 3'.

Amplification by PCR, utilizing the primers, and template, was with a Cetus-Perkin-Elmer PCR kit, and was performed according to the manufacturer's directions. The PCR conditions were 29 cycles of 94°C for a minute, 37°C for 2 minutes, 72°C for 3 minutes; and the final incubation was at 72°C for 10 minutes. The DNA can be stored at 4°C or -20°C overnight.

After amplification, the PCR products were digested with HindIII and Sall. The major product of 1.1 kb was purified by electrophoresis on a gel, and the eluted purified product was ligated with a large Sall-HindIII fragment of pBR322. In order to isolate correct recombinants, competent HB101 cells were transformed with the recombinant vectors, and after cloning, the desired recombinants were identified on the basis of the predicted size of HindIII-Sall fragments excised from the clones. One of the clones which contained the a HindIII-Sall fragment of the correct size was named pBR322/C100<sup>d</sup>. Confirmation that this clone contained amplified C100 was by direct sequence analysis of the HindIII-Sall fragment.

The expression vector containing C100 was constructed by ligating the HindIII-Sall fragment from pBR322/C100<sup>d</sup> to a 13.1 kb BamHI-Sall fragment of pBS24.1, and a 1369 bp BamHI-HindIII fragment containing the ADH2/GAP promoter. (The latter fragment is described in EPO 164,556). The pBS24.1 vector is described in commonly owned U.S.S.N. 382,805 filed 19 July 1989. The ADH2/GAP promoter fragment was obtained by digestion of the vector pGAP/AG/HindIII with HindIII and BamHI, followed by purification of the 1369 bp fragment on a gel.

Competent HB101 cells were transformed with the recombinant vectors; and correct recombinants were identified by the generation of a 2464 bp fragment and a 13.1 kb fragment generated by BamHI and Sall diges-

tion of the cloned vectors. One of the cloned correct recombinant vectors was named pC100<sup>-</sup>d#3.

In order to express C100, competent cells of *Saccharomyces cerevisiae* strain AB122 (MATa leu2 ura3-53 prb 1-1122 pep4-3 prc1-407[cir-0]) were transformed with the expression vector pC100<sup>-</sup>d#3. The transformed cells were plated on URA-sorbitol, and individual transformants were then streaked on Leu<sup>-</sup> plates.

Individual clones were cultured in Leu<sup>-</sup>, ura<sup>-</sup> medium with 2% glucose at 30°C for 24-36 hours. One liter of Yeast Extract Peptone Medium (YEP) containing 2% glucose was inoculated with 10 ml of the overnight culture, and the resulting culture was grown at 30°C at an agitation rate of 400 rpm and an aeration rate of 1 L of air per 1 L of medium per minute (i.e., 1vvm) for 48 hours. The pH of the medium was not controlled. The culture was grown in a BioFlo II fermentor manufactured by New Brunswick Science Corp. Following fermentation, the cells were isolated and analyzed for C100 expression.

Analysis for expressed C100 polypeptide by the transformed cells was performed on total cell lysates and crude extracts prepared from single yeast colonies obtained from the Leu<sup>-</sup> plates. The cell lysates and crude extracts were analyzed by electrophoresis on SDS polyacrylamide gels, and by Western blots. The Western blots were probed with rabbit polyclonal antibodies directed against the SOD-C100 polypeptide expressed in yeast. The expected size of the C100 polypeptide is 364 amino acids. By gel analysis the expressed polypeptide has a MW, of 39.9K.

Both analytical methods demonstrated that the expressed C100 polypeptide was present in total cell lysates, but was absent from crude extracts. These results suggest that the expressed C100 polypeptide may be insoluble.

### Example 3: Expression of HCV Antigen S2

HCV antigen S2 contains a sequence from the hydrophobic N-terminus of the S domain. It includes amino acids 199-328 of Figure 1.

The protocol for the construction of the expression vector encoding the S2 polypeptide and for its expression in yeast was analogous to that used for the expression of the C100 polypeptide, described in Example 2.

The template for the PCR reaction was the vector pBR322/Pi14a, which had been linearized by digestion with HindIII. Pi14a is a cDNA clone that encodes amino acids 199-328.

The oligonucleotides used as primers for the amplification by PCR of the S2 encoding sequence were the following.

For the 5'-region of the S2 sequence:

5' GAG TGC TCA AGC TTC AAA ACA AAA TGG GGC TCT  
ACC ACG TCA CCA ATG ATT GCC CTA AC 3';

and

for the 3'-region of the S2 sequence:

5'GAG TGC TCG TCG ACT CAT TAA GGG GAC CAG TTC  
ATC ATC ATA TCC CAT GCC AT 3'.

The primer for the 5'-region introduces a HindIII site and an ATG start codon into the amplified product. The primer for the 3'-region introduces translation stop codons and a SalI site into the amplified product.

The PCR conditions were 29 cycles of 94°C for a minute, 37°C for 2 minutes, 72°C for 3 minutes, and the final incubation was at 72°C for 10 minutes.

The main product of the PCR reaction was a 413 bp fragment, which was gel purified. The purified fragment was ligated to the large fragment obtained from pBR322 digested with HindIII and SalI fragment, yielding the plasmid pBR322/S2d.

Ligation of the 413 bp HindIII-SalI S2 fragment with the 1.36 kb BamHI-HindIII fragment containing the ADH2/GAP promoter, and with the large BamHI-SalI fragment of the yeast vector pBS24.1 yielded recombinant vectors, which were cloned. Correct recombinant vectors were identified by the presence of a 1.77 kb fragment after digestion with BamHI and SalI. An expression vector constructed from the amplified sequence, and containing the sequence encoding S2 fused directly to the ADH2/GAP promoter is identified as pS2d#9.



Example 4: Synthesis of HCV C Antigen

HCV antigen C22 is from the C domain. It comprises amino acids 1-122 of Figure 1.

5 The protocol for the construction of the expression vector encoding the C polypeptide and for its expression in yeast was analogous to that used for the expression of the C100 polypeptide, described supra, except for the following.

The template for the PCR reaction was pBR322/ Ag30a which had been linearized with HindIII. Ag30 is a cDNA clone that encodes amino acids 1-122. The oligonucleotides used as primers for the amplification by PCR of the C encoding sequence were the following.

10 For the 5'-region of the C sequence:

5' GAG TGC AGC TTC AAA ACA AAA TGA GCA CGA  
ATC CTA AAC CTC AAA AAA AAA AC 3',

and

20 for the 3'-region of the C sequence:

5' GAG TGC TCG TCG ACT CAT TAA CCC AAA TTG CGC  
GAC CTA CGC CGG GGG TCT GT 3'.

25 The primer for the 5'-region introduces a HindIII site into the amplified product, and the primer for the 3'-region introduces translation stop codons and a Sall site. The PCR was run for 29 cycles of 94°C for a minute, 37°C for 2 minutes, 72°C for 3 minutes, and the final incubation was at 72°C for 10 minutes.

The major product of PCR amplification is a 381 bp polynucleotide. Ligation of this fragment with the Sall-HindIII large Sall-HindIII fragment of pBR322 yielded the plasmid pBR322/C2.

30 Ligation of the 381 bp HindIII-Sall C coding fragment excised from pBR322/C2 with the 1.36 kb BamHI-HindIII fragment containing the ADH2/GAP promoter, and with the large BamHI-Sall fragment of the yeast vector pBS24.1 yielded recombinant vectors, which were cloned. Correct recombinant vectors were identified by the presence of a 1.74 kb fragment after digestion with BamHI and Sall. An expression vector constructed from the amplified sequence, and containing the sequence encoding C fused directly to the ADH2/GAP promoter is identified as pC22.

35 Analysis for expressed C polypeptide by the transformed cells was performed on total cell lysates and crude extracts prepared from single yeast colonies obtained from the Leu<sup>-</sup> plates. The cell lysates and crude extracts were analyzed by electrophoresis on SDS polyacrylamide gels. The C polypeptide is expected to have 122 amino acids and by gel analysis the expressed polypeptide has a MW<sub>r</sub> of approximately 13.6 Kd.

Example 5: Synthesis of NS5 Polypeptide

45 This polypeptide contains sequence from the N-terminus of the NS5 domain. Specifically it includes amino acids 2054 to 2464 of Figure 1. The protocol for the construction of the expression vector encoding the NS5 polypeptide and for its expression were analogous to that used for the expression of C33c (see Example 1).

Example 6: Radioimmunoassay (RIA) for Antibodies to HCV

50 The HCV antigens of Examples 1-5 were tested in an RIA format for their ability to detect antibodies to HCV in the serum of individuals clinically diagnosed as having HCV (Non-A, Non-B) and in serum from blood given by paid blood donors.

55 The RIA was based upon the procedure of Tsu and Herzenberg (1980) in SELECTED METHODS IN CELLULAR IMMUNOLOGY (W.H. Freeman & Co.), pp. 373-391. Generally, microtiter plates (Immulon 2, Removawell strips) are coated with purified HCV antigen. The coated plates are incubated with the serum samples or appropriate controls. During incubation, antibody, if present, is immunologically bound to the solid phase antigen. After removal of the unbound material and washing of the microtiter plates, complexes of human antibody-NANBV antigen are detected by incubation with <sup>125</sup>I-labeled sheep anti-human immunoglobulin. Unbound labeled antibody is removed by aspiration, and the plates are washed. The radioactivity in individual wells is

determined; the amount of bound human anti-HCV antibody is proportional to the radioactivity in the well.

Specifically, one hundred microliter aliquots containing 0.1 to 0.5 micrograms of the HCV antigen in 0.125 M Na borate buffer, pH 8.3, 0.075 M NaCl (BBS) was added to each well of a microliter plate (Dynatech Immulon 2 Removawell Strips). The plate was incubated at 4°C overnight in a humid chamber, after which, the antigen solution was removed and the wells washed 3 times with BBS containing 0.02% Triton X-100 (BBST). To prevent non-specific binding, the wells were coated with bovine serum albumin (BSA) by addition of 100 microliters of a 5 mg/ml solution of BSA in BBS followed by incubation at room temperature for 1 hour; after this incubation the BSA solution was removed. The antigens in the coated wells were reacted with serum by adding 100 microliters of serum samples diluted 1:100 in 0.01M Na phosphate buffer, pH 7.2, 0.15 M NaCl (PBS) containing 10 mg/ml BSA, and incubating the serum containing wells for 1 hr at 37°C. After incubation, the serum samples were removed by aspiration, and the wells were washed 5 times with BBST. Antibody bound to the antigen was determined by the binding of <sup>125</sup>I-labeled F(ab)<sub>2</sub> sheep anti-human IgG to the coated wells. Aliquots of 100 microliters of the labeled probe (specific activity 5-20 microcuries/microgram) were added to each well, and the plates were incubated at 37°C for 1 hour, followed by removal of excess probe by aspiration, and 5 washes with BBST. The amount of radioactivity bound in each well was determined by counting in a counter which detects gamma radiation.

Table 1 below presents the results of the tests on the serum from individuals diagnosed as having HCV.

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Table 1

	<u>INDIVIDUAL</u>	<u>ANTIGEN</u>				
		<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u>	<u>NS5</u>
5	CVH IVDA	P	P	P(+++)	P	P
	CVH IVDA	P	P	P(+)	P	P
10	CVH IVDA	P	P	P(+)	P	P
	CVH NOS	P	P	P	P	P
	AVH NOS HS	N	N	N	N	N
	AVH NOS HS	P	N	N	N	N
15	AVH NOS HS	P	N	N	N	N
	AVH NOS HS	P/N	N	N	N	N
	AVH PTVH	N	N	N	P/N	N
	AVH NOS HS	N	N	N	N	N
20	AVH NOS	N	N	N	N	P
	AVH PTVH	N	N	N	N	N
	AVH IVDA	N	P	N	P	P
25	AVH PTVH	P	P/N	N	N	P
	AVH NOS	N	P	N	N	N
	AVH IVDA	N	P	N	P	P
	AVH NOS HS	P/N	N	N	N	N
30	AVH PTVH	N	N	N	N	N
	CVH IVDA	P	P	P	P	P
	CVH IVDA	P	P	P	P	P
35	AVH NOS HS	N	N	N	N	N
	CVH PTVH	P	P	N	N	N
	AVH PTVH	P	N	P(+)	P(+++)	N
	CVH PTVH	N	P	P	P	P
40	CVH NOS HS	P	P	P	P	N
	CVH NOS	N	P	P/N	P	P

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<u>INDIVIDUAL</u>		<u>ANTIGEN</u>			
		<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u> <u>NS5</u>
5	CVH IVDA	N	N	N	P   N
	AVH IVDA	P	P	P	P   P
	AVH IVDA	P	P	P	P   P
10	CVH IVDA	P	P	P	P   P
	AVH IVDA	P/N	P	N	P   P
	AVH IVDA	N	P	P	P   N
15	CVH PTVH	P	P/N	N	N   N
	CVH NOS	N	N	N	N   N
	CVH NOS	N	N	N	N   N
20	CVH IVDA	P	P	P	P   P
	AVH IVDA	P	P	P	P   P
	CVH PTVH	P	P	P	P   P
25	AVH PTVH?	N	P	P	P   P
	AVH IVDA	N	P	N	P   N
	AVH NOS	N	N	N	N   N
30	AVH NOS	N	N	N	N   N
	CVH NOS	N	P	N	N   P
	CVH NOS	P	P	N	N   N
35	CVH NOS HS	P	P	P	P   P
	CVH PTVH	P	P	N	P   P
	AVH nurse	P	P	N	N   N
40	AVH IVDA	P	P	P	P   N
	AVH IVDA	N	P	P(+)	P(+++)   N
	AVH nurse	P/N	P	N	N   N
45	AVH PTVH	P/N	P	P	N   P
	AVH NOS	N	P/N	N	N   P
	AVH NOS	N	P	N	P   N
50	AVH PTVH	P	P/N	N	N   N
	AVH PTVH	N	P	N	P   P
	AVH PTVH	P	P	P	P   P
55	AVH PTVH	N	P	N	N   P
	CVH PTVH	P/N	P	P(+)	P(+++)   N
	AVH PTVH	N	P/N	P(+)	P(+++)   P

<u>INDIVIDUAL</u>		<u>ANTIGEN</u>			
		<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u> <u>NS5</u>
	AVH PTVH	P	(?)	P	N   P
5	CVH PTVH	N	P	N	P   P
	CVH PTVH	N	P	P	P   P
	CVH PTVH	N	N	N	N   N
10	AVH NOS	N	N	N	N   N
	AVH nurse	P	P	N	N   N
	CVH PTVH	N	P	N	N   P
	AVH IVDA	N	P	N	P/N   N
15	CVH PTVH	P	P	P(+)	P(+++)   P
	AVH NOS	P	P	N	N   N
	AVH NOS	P/N	P	N	N   P
	AVH PTVH	P/N	P	P	P   P
20	AVH NOS	N	P	P	P   P/N
	AVH IVDA	N	P	N	N   P
	AVH NOS	N	P/N	N	N   N
25	AVH NOS	P	P	N	N   P
	AVH PTVH	N	P	P	P   P
	crypto	P	P	P	P   P
	CVH NOS	N	P	P	P   P
30	CVH NOS	N	N	N	N   N
	AVH PTVH	N	P	P(+)	P(++)   N
	AVH PTVH	N	P/N	P(+)	P(++)   P
35	AVH PTVH	N	P/N	P(+)	P(++)   P
	CVH IVDA	P	P	P	P   P
	CVH IVDA	P	P	P	P   P
	CVH IVDA	P	P	P	P   P
40	CVH IVDA	P	P	P	P   P
	AVH NOS	N	P	N	N   N
	CVH IVDA	P	P	P	P   P/N
	AVH IVDA	P	P	P	P   N
45	AVH NOS	P	P	N	N   N
	AVH NOS	P	P	N	N   N
	CVH PTVH	P	P	N	N   P/N
50					

55

<u>INDIVIDUAL</u>		<u>ANTIGEN</u>			
		<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u> <u>NS5</u>
	AVH PTVH	N	P	N	P P
5	AVH NOS	N	N	N	N N
	AVH NOS	N	P	N	N N
	AVH NOS	P	N	N	N N
10	CVH NOS	N	N	N	N N
	AVH NOS	N	P/N	N	N N
	AVH IVDA	N	P	P	P P
	crypto	N	P	N	N P/N
15	crypto	P	P	P/N	P P
	AVH IVDA	N	N	P	P N
	AVH IVDA	N	P	P	P N
	AVH NOS	N	N	N	N N
20	AVH NOS	N	N	N	N N
	CVH IVDA	P	P	P	P P
	CVH PTVH	N	N	N	N N
25	CVH PTVH	P	P	P(+)	P(+++) P
	CVH PTVH	P	P	P(+)	P(+++) P
	CVH NOS	P/N	N	N	N N
	CVH NOS	N	N	N	N N
30	CVH PTVH	P	P	P	P P
	CVH PTVH	P	P	P	P P
	CVH PTVH	P	P	P	P P
35	AVH IVDA	N	P	P	P P
	CVH NOS	N	N	N	N N
	CVH NOS	N	N	N	N N
	CVH PTVH	P	P	P	P P
40	AVH NOS	P	P	N	N P/N
	AVH NOS	N	P/N	N	N N
	CVH PTVH	P	P	N	N P
	CVH NOS	N	P/N	N	N N
45	AVH NOS	N	P	N	N N
	AVH NOS	N	P	N	N N
	CVH PTVH	N	P	N	N N
50					

<u>INDIVIDUAL</u>		<u>ANTIGEN</u>			
		<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u> <u>NS5</u>
	AVH IVDA	N	P	N	P    P
5	AVH NOS	P	N	N	N    N
	CVH NOS	N	N	N	N    N
	CVH NOS	N	N	N	N    N
	CVH IVDA	P	P	P	P    P
10	CVH IVDA	P/N	P	P	P    P
	CVH IVDA	P	P	P	P    P
	CVH IVDA	N	P	P	P    P
15	AVH NOS	N	P	N	N    N
	CVH IVDA	N	P	N	N    P
	CVH IVDA	N	P	N	N    P
	AVH PTVH	P	P	N	P    P
20	AVH PTVH	P	P	N	P    P
	CVH NOS	N	P/N	N	N    P/N
	CVH NOS	N	P	N	N    N
25	CVH NOS	N	N	N	N    N
	CVH PTVH	P	P	P	P    P
	CVH PTVH	P	P	P	P    P
	CVH PTVH	P	P	P	P    P
30	AVH IVDA	N	P	N	N    P
	AVH IVDA	N	P	P(++)	P(+)    P
	CVH PTVH	P	P	P	P    P
35	AVH PTVH	N	P	P	P    P
	CVH PTVH?	N	P	P	P    P
	CVH PTVH?	P/N	P	P	P    P
	CVH NOS HS	P	P	N	N    N
40	CVH IVDA	P	P	P	P    N
	CVH PTVH	N	P	P	P    P
	CVH PTVH	P	P	P	P    P/N
	CVH NOS	P	P	P	P    P
45	CVH IVDA	P	P	P	P    P
	CVH PTVH	P	P	P	P    N
	CVH PTVH	P	P	P	P    P
50					

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	<u>INDIVIDUAL</u>			<u>ANTIGEN</u>	
	<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u>	<u>NS5</u>
	CVH NOS	N	N	N	P/N
5	CVH NOS	N	P/N	N	P/N
	CVH PTVH	P	P	P	P
	CVH NOS	N	P	N	N
10	CVH NOS	N	N	N	N
	CVH NOS	P	P	N	P/N
	CVH NOS	N	N	N	N
	CVH NOS HS	P	P	P	P
15	CVH NOS HS	P	P	P	P
	CVH PTVH	N	N	N	N
	AVH PTVH	N	P	P	P
	AVH NOS		-	-	
20	CVH PTVH	N	P	P(+++)	N
	crypto	P	P	P	P
	crypto	P	P	P	P
25	crypto	N	P	N	N
	crypto	N	P	P	P
	CVH PTVH	P	P	P	P
	crypto	N	N	N	N
30	crypto	N	P	N	P/N
	crypto	N	P	N	P
	crypto	P	P	P	P
35	crypto	N	P	P	N
	crypto		-	-	
	crypto		-	-	
	CVH NOS		-	-	
40	AVH-IVDA	N	P	P(+)	P

45

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<u>INDIVIDUAL</u>	<u>ANTIGEN</u>				
	<u>S2</u>	<u>C22</u>	<u>C100</u>	<u>C33c</u>	<u>NS5</u>
AVH-IVDA	N	P/N	N	P(++)	N

AVH = acute viral hepatitis

CVH = chronic viral hepatitis

PTVH = post-transfusion viral hepatitis

IVDA = intravenous drug abuser

crypto = cryptogenic hepatitis

NOS = non-obvious source

P = positive

N = negative

Per these results, no single antigen reacted with all sera. C22 and C33c were the most reactive and S2 reacted with some sera from some putative acute HCV cases with which no other antigen reacted. Based on these results, the combination of two antigens that would provide the greatest range of detection is C22 and C33c. If one wished to detect a maximum of acute infections, S2 would be included in the combination.

Table 2 below presents the results of the testing on the paid blood donors.

Table 2

<u>Donor</u>	<u>Antigens</u>				
	<u>C100</u>	<u>C33c</u>	<u>C22</u>	<u>S2</u>	<u>NS5</u>
1	N	N	N	N	N
2	N	N	N	N	N
3	P	P	P	P	P
4	N	N	N	N	N
5	N	N	N	N	N
6	N	N	N	N	N
7	N	N	N	N	N
8	N	N	N	N	N

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		Antigens				
	<u>Donor</u>	<u>C100</u>	<u>C33c</u>	<u>C22</u>	<u>S2</u>	<u>NS5</u>
5	9	N	N	N	N	N
	10	N	N	N	N	N
	11	N	N	N	N	N
	12	N	N	N	N	N
10	13	N	N	N	N	N
	14	N	N	N	N	N
	15	N	N	N	N	N
	16	N	N	N	N	N
15	17	N	N	N	N	N
	18	P	P	P	P	P
	19	P	P	N	P	P
	20	P	P	N	P	P
20	21	N	N	N	N	N
	22	N	P	P	N	P
	23	P	P	P	P	P
	24	N	N	N	N	N
25	25	N	N	N	N	N
	26	N	N	N	N	N
	27	N	N	N	N	N
	28	N	N	N	N	N
30	29	N	N	N	N	N
	30	N	N	N	N	N
	31	P	P	P	N	P
	32	N	N	N	N	N
35	33	N	N	N	N	N
	34	N	N	N	N	P
	35	N	N	P	N	P
	36	N	N	N	N	N
40	37	N	N	N	N	N
	38	N	N	N	N	N
	39	N	N	N	N	N
	40	N	N	N	N	N
45	41	N	N	N	N	P
	42	N	N	N	N	N

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		Antigens				
	<u>Donor</u>	<u>C100</u>	<u>C33c</u>	<u>C22</u>	<u>S2</u>	<u>NS5</u>
5	43	N	N	N	N	N
	44	N	N	N	N	N
	45	N	N	N	N	N
	46	N	N	N	N	N
10	47	P	P	N	N	P
	48	N	N	N	N	N
	49	N	N	N	N	N
	50	N	N	N	N	N
15	51	N	P	P	N	P
	52	N	N	N	N	N
	53	N	P	P	N	P
	54	P	P	P	P	N
20	55	N	N	N	N	N
	56	N	N	N	N	N
	57	N	N	N	N	N
	58	N	N	N	N	N
25	59	N	N	N	N	N
	60	N	N	N	N	N
	61	N	N	N	N	N
	62	N	N	N	N	N
30	63	N	N	N	N	N
	64	N	N	N	N	N
	65	N	N	N	N	N
	66	N	N	N	N	N
35	67	N	N	N	N	N
	68	N	N	N	N	N
	69	N	N	N	N	N
	70	P	P	P	P	P
40	71	N	N	N	N	N
	72	N	N	N	N	N
	73	P	P	P	P	N
	74	N	N	N	N	N
45	75	N	N	N	N	N
	76	N	N	N	N	P

55

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		Antigens				
	<u>Donor</u>	<u>C100</u>	<u>C33c</u>	<u>C22</u>	<u>S2</u>	<u>NS5</u>
5	77	N	N	N	N	N
	78	N	N	N	N	N
	79	N	N	N	N	N
	80	N	N	N	N	N
10	81	N	N	N	N	N
	82	N	N	N	N	N
	83	P	P	N	N	N
15	84	N	N	P	N	N
	85	N	N	N	N	N
	86	P	P	P	P	N
	87	N	N	N	N	N
20	88	N	N	N	N	N
	89	P	P	P	P	P
	90	P	P	P	P	N
25	91	N	N	N	N	P
	92	P	P	P	N	N
	93	N	N	N	N	N
	94	N	N	N	N	N
30	95	N	N	N	N	N
	96	N	N	N	N	N
	97	N	N	N	N	N
35	98	N	P	P	P	P
	99	P	P	P	P	P
	100	N	N	N	N	N
	101	P	P	P	P	P
40	102	N	N	N	N	N
	103	N	N	N	N	N
	104		N	N	N	N
45	105	P	P	P	P	N
	106	N	N	N	N	N
	107	N	N	N	N	N
	108	N	N	N	N	N
50	109	P	P	P	P	P
	110	P	P	P	N	P

55

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		Antigens				
	<u>Donor</u>	<u>C100</u>	<u>C33c</u>	<u>C22</u>	<u>S2</u>	<u>NS5</u>
5	111	P	P	P	N	P
	112	N	N	N	N	N
	113	P	P	P	P	P
	114	N	N	N	N	N
10	115	N	N	N	N	N
	116	P	P	P	P	P
	117	N	N	N	N	N
	118	N	N	N	N	N
15	119	N	N	N	N	N
	120	P	P	P	P	P
	121	N	N	N	N	N
20	122	N	P	P	N	P
	123	N	N	N	N	N
	124	N	N	N	N	N
	125	N	N	N	N	N
25	126	P	N	N	N	N
	127	N	N	N	N	N
	128	N	N	N	N	N
30	129	N	N	N	N	N
	130	P	P	P	P	N
	131	N	N	N	N	P
	132	N	N	N	N	N
35	133	N	N	N	N	N
	134	N	N	N	N	N
	135	N	N	N	N	N
	136	N	N	N	N	N
40	137	N	N	N	N	N
	138	N	N	N	N	N
	139	N	N	N	N	N
45	140	P	N	N	N	N
	141	P	N	P	P	P
	142	N	N	N	N	N
	143	N	N	N	N	N
50	144	N	N	N	N	N

55

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		Antigens				
	<u>Donor</u>	<u>C100</u>	<u>C33c</u>	<u>C22</u>	<u>S2</u>	<u>NS5</u>
5	145	N	N	N	N	N
	146	N	N	N	N	N
	147	N	N	N	N	N
	148	N	N	N	N	N
10	149	N	N	N	N	N
	150	N	N	N	N	N
	151	N	N	N	N	N
	152	N	N	N	N	N
15	153	N	N	N	N	N
	154	P	P	P	P	P
	155	N	N	N	N	N
	156	N	N	N	N	N
20	157	N	N	N	N	N
	158	N	N	N	N	N
	159	N	N	N	N	N
	160	N	N	N	N	N
25	161	P	P	P	P	P
	162	N	N	N	N	N
	163	N	N	N	N	N
	164	P	P	P	N	P
30	165	N	N	N	N	N
	166	P	P	P	N	P
	167	N	N	N	N	N
	168	N	N	N	N	N
35	169	N	N	N	N	N
	170	N	N	N	N	N
	171	N	N	N	N	N
	172	N	N	N	N	N
40	173	N	N	N	N	N
	174	N	N	N	N	N
	175	N	N	N	N	N
	176	N	N	N	N	N
45	177	N	N	N	N	P
	178	N	N	N	N	N

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		Antigens				
	Donor	C100	C33c	C22	S2	NS5
5	179	N	N	N	N	N
	180	N	N	N	N	N
	181	N	N	N	N	N
	182	N	N	N	N	N
10	183	P	P	P	P	P
	184	N	N	N	N	N
	185	N	N	N	N	N
	186	N	N	N	N	N
15	187	N	N	N	N	N
	188	N	P	P	N	N
	189	N	N	N	N	N
	190	N	N	N	N	N
20	191	N	N	N	N	N
	192	N	N	N	N	N
	193	N	N	N	N	N
	194	N	N	N	N	N
25	195	N	N	N	N	N
	196	N	N	N	N	N
	197	N	N	N	N	P
	198	P	P	P	N	N
30	199	N	N	N	N	P
	200	P	P	P	P	N

The results on the paid donors generally confirms the results from the sera of infected individuals.

#### Example 7: ELISA Determinations of HCV Antibodies Using Combination of HCV Antigens

Plates coated with a combination of C22 and C33c antigens are prepared as follows. A solution containing coating buffer (50mM Na Borate, pH 9.0), 21 ml/plate, BSA (25 micrograms/ml), C22 and C33c (2.50 micrograms/ml each) is prepared just prior to addition to the Removeawell Immulon I plates (Dynatech Corp.). After mixing for 5 minutes, 0.2ml/well of the solution is added to the plates, they are covered and incubated for 2 hours at 37°C, after which the solution is removed by aspiration. The wells are washed once with 400 microliters wash buffer (100 mM sodium phosphate, pH 7.4, 140 mM sodium chloride, 0.1% (W/V) casein, 1% (W/V) Triton x-100, 0.01% (W/V) Thimerosal). After removal of the wash solution, 200 microliters/well of Postcoat solution (10 mM sodium phosphate, pH 7.2, 150 mM sodium chloride, 0.1% (w/v) casein, 3% sucrose and 2 mM phenylmethylsulfonylfluoride (PMSF)) is added, the plates are loosely covered to prevent evaporation, and are allowed to stand at room temperature for 30 minutes. The wells are then aspirated to remove the solution, and lyophilized dry overnight, without shelf heating. The prepared plates may be stored at 2-8°C in sealed aluminum pouches with dessicant (3 g Sorb-it™ packs).

In order to perform the ELISA determination, 20 microliters of serum sample or control sample is added to a well containing 200 microliters of sample diluent (100 mM sodium phosphate, pH 7.4, 500 mM sodium chloride, 1 mM EDTA, 0.1% (W/V) Casein, 0.01% (W/V) Thimerosal, 1% (W/V) Triton X-100, 100 micrograms/ml yeast extract). The plates are sealed, and are incubated at 37°C for two hours, after which the solution is removed by aspiration, and the wells are washed three times with 400 microliters of wash buffer (phosphate buffered saline (PBS) containing 0.05% Tween 20). The washed wells are treated with 200 microliters of mouse anti-human-IgG-horse radish peroxidase (HRP) conjugate contained in a solution of Ortho conjugate diluent

(10 mM sodium phosphate, pH 7.2, 150 mM sodium chloride, 50° (V/V) fetal bovine serum, 1% (V/V) heat treated horse serum, 1 mM  $K_3Fe(CN)_6$ , 0.05% (W/V) Tween 20, 0.02% (W/V) Thimerosal). Treatment is for 1 hour at 37°C, the solution is removed by aspiration, and the wells are washed three times with 400 ml wash buffer, which is also removed by aspiration. To determine the amount of bound enzyme conjugate, 200 microliters of substrate solution (10 mg O-phenylenediamine dihydrochloride per 5 ml of Developer solution) is added. Developer solution contains 50 mM sodium citrate adjusted to pH 5.1 with phosphoric acid, and 0.6 microliters/ml of 30%  $H_2O_2$ . The plates containing the substrate solution are incubated in the dark for 30 minutes at room temperature, the reactions are stopped by the addition of 50 microliters/ml 4N sulfuric acid, and the ODs determined.

In a similar manner, ELISAs using fusion proteins of C22 and C33c, and C22, C33c, and S2 and combinations of C22 and C100, C22 and S2, C22 and an NS5 antigen, C22, C33c, and S2, and C22, C100, and S2 may be carried out.

Modifications of the above-described modes for carrying out the invention that are obvious to those of skill in the fields of molecular biology, immunology, and related fields are intended to be within the scope of the following claims.

#### Claims

1. A combination of synthetic hepatitis C viral (HCV) antigens comprising:
  - (a) a first HCV antigen from the C domain; and
  - (b) at least one additional HCV antigen selected from the group consisting of
    - (i) an HCV antigen from the NS3 domain;
    - (ii) an HCV antigen from the NS4 domain;
    - (iii) an HCV antigen from the S domain; and
    - (iv) an HCV antigen from the NS5 domain.
2. A combination of synthetic hepatitis C viral (HCV) antigens comprising:
  - (a) a first HCV antigen consisting essentially of the C domain; and
  - (b) a second HCV antigen from the NS3 domain.
3. The combination of claim 2 wherein the first HCV antigen is C22 and the second HCV antigen is C33c.
4. The combination of claim 2 including (c) a third HCV antigen from the S domain.
5. The combination of claim 3 including (c) HCV antigen S2.
6. A combination of synthetic HCV antigens comprising:
  - (a) a first HCV antigen consisting essentially of the C domain; and
  - (b) a second HCV antigen from the NS4 domain.
7. The combination of claim 6 wherein the first HCV antigen is C22 and the second HCV antigen is C100.
8. The combination of claim 6 including (c) a third HCV antigen from the S domain.
9. The combination of claim 7 including (c) HCV antigen S2.
10. The combination of claim 1, 2, 3, 4, 5, 6, 7, 8 or 9 wherein the combination is in the form of a fusion polypeptide.
11. The combination of claim 1, 2, 3, 4, 5, 6, 7, 8 or 9 wherein the combination is in the form of said first HCV antigen and said additional antigens individually bound to a common solid matrix.
12. The combination of claim 11 wherein the solid matrix is the surface of a microtiter plate well, a bead or a dipstick.
13. The combination of claim 1, 2, 3, 4, 5, 6, 7, 8 or 9 wherein the combination is in the form of a mixture of said first HCV antigen and said additional HCV antigen(s).



14. A method for detecting antibodies to hepatitis C virus (HCV) in a mammalian body component suspected of containing said antibodies comprising contacting said body component with the combination of synthetic HCV antigens of claim 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 under conditions that permit antibody-antigen reaction and detecting the presence of immune complexes of said antibodies and said antigens.

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15. A method for detecting antibodies to hepatitis C virus (HCV) in a mammalian body component suspected of containing said antibodies comprising contacting said body component with a panel of synthetic HCV antigens comprising:

(a) a first HCV antigen from the C domain; and

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(b) at least one additional HCV antigen selected from the group consisting of

(i) an HCV antigen from the NS3 domain;

(ii) an HCV antigen from the NS4 domain;

(iii) an HCV antigen from the S domain; and

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(iv) an HCV antigen from the NS5 domain under conditions that permit antibody-antigen reaction and detecting the presence of immune complexes of said antibodies and said antigens.

16. A kit for carrying out an assay for detecting antibodies to hepatitis C antigen (HCV) in a mammalian body component suspected of containing said antibodies comprising in packaged combination:

(a) the combination of synthetic HCV antigens of any one of claims 1-13;

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(b) standard control reagents; and

(c) instructions for carrying out the assay.

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-341 GGGAGCCCCCTGATGGGGGCGA  
CGGTGGGGGACTACCCCGCT

-319 CACTCCACCATGAATCACTCCCTTGTGAGGAACTACTCTCTTCACCCAGAAAGCGTCTAG  
GTGAGGTGGTACTTAGTGAGGGGACACTCTCTGATGACAGAAGTSCGTCTTTCGCAGATC

-359 CCATGGCGTTAGTATGASTGTCTGTCAGCCTCCAGGACCCCTCCCGGGAGAGCCATA  
GGTACCCCAATCATACTCACAGCACCTCGGAGGTCTTGGGGGGAGGGCCCTCTCGGTAT

-199 GTGGTCTGGGAACCGGTGAGTACACCGGAATTGTCAGGACGACCGGGTCTTTCTTGGG  
CACCAGACGCTTGGCCACTCATGTGGCTTAACGGTCTGCTGGCCAGGAAGAACCT

-139 TCAACCCCTCAATGCCCTGGAGATTGGGGCTGCCCCGCAAGACTGCTAGCCSAGTAGT  
AGTTGGGCGAGTTACGGACCTCTAAACCCGACGGGGCTTCTGACGATCGGCTCATCA

- 79 GTTGGGTCCGSAAGGCCCTTGTGGTACTGCCCTGATAGGGTGCTTGGAGTGGCTCGGGAG  
CAACCCAGCGCTTTCGGGAACCATGACGGACTATCCACGAACGCTCACGGGGCCCTC

- 19 GTCTCTAGACCGTGCACC  
CAGAGCATCTGCCACGTGG

Arg Thr

MetSerThrAsnProLysProGlnLysLysAsnLysArgAsnThrAsnArgArgProGln  
1 ATGAGCACGAATCCTAACCTCAAAAAAAAAAAACAAACGTAACACCAACCGTCCGCCACAG  
TACTCGTGCTTAGGATTGGAGTTTTTTTTTTGTTTGCATTGTGGTGGCAGCGGGTGTC

61 AspValLysPheProGlyGlyGlyGlnIleValGlyGlyValTyrLeuLeuProArgArg  
GACGTCAAGTTCCCGGGTGGCGGTGAGATCGTTGGTGGAGTTTACTTGTTCGCCGACG  
CTGCAGTTCAAGGGCCACCGCCAGTCTAGCAACCACTCAAATGAACAACGGCGCTCC

121 GlyProArgLeuGlyValArgAlaThrArgLysThrSerGluArgSerGlnProArgGly  
GGCCCTAGATTGGGTGTGCGCGGACGAGAAAGACTTCCGAGCGGTGCAACCTCGAGGT  
CCGGGATCTAACCCACACGCGCGCTCTCTTTCTGAAGGCTCGCCAGCGTTGGAGCTCCA

181 ArgArgGlnProIleProLysAlaArgArgProGluGlyArgThrTrpAlaGlnProGly  
AGACGTGAGCTATCCCCAAGGCTCGTCCGCCCGAGGGCAGGACCTGGGCTCAGCCCGGG  
TCTGCAGTCGGA TAGGGGTTCGAGCAGCCCGGCTCCGCTCTGGACCCGAGTCSGGCCC

241 TyrProTrpProLeuTyrGlyAsnGluGlyCysGlyTrpAlaGlyTrpLeuLeuSerPro  
TACCTTGGCCCCCTCTATGGCAATGAGGGCTGCGGGTGGCGGGATGGCTCTCTCTCC  
ATGGGAACCGGGAGATACCGTACTCCGACGCCACCCTCCCTACCGAGGACAGAGGG

301 ArgGlySerArgProSerTrpGlyProThrAspProArgArgArgSerArgAsnLeuGly  
CGTGCTCTCGGCC TAGCTGGGGCCCCACAGACCCCGGCTAGGTGCGCAATTGGGT  
GCACCCAGAGCCGGATCGACCCCGGGGTGTCTGGGGGCTCATCCAGCGCTTAACCCA

361 LysValIleAspThrLeuThrCysGlyPheAlaAspLeuMetGlyTyrIleProLeuVal  
AAGGTATCGATACCTTACGTGCGGCTTCCGCGACCTCATGGGGTACATACCGCTCGTC  
TTCCASTAGCTATGGGAATGTACGCCGAAGCGGCTGGAGTACCCCATGTATGGCGAGCAG

421 GlyAlaProLeuGlyGlyAlaAlaArgAlaLeuAlaHisGlyValArgValLeuGluAsp  
GGCCCTCTCTTGGAGGGCGCTGCGAGGCCCTGGCGCATGCGGTCCGGGTCTTGGGAAGAC  
CCGCGGGGAGAACCTCCGCGACGTTCCGGGACCGGTACCGCAGGCCCAAGACCTCTCTG

Thr

481 GlyValAsnTyrAlaThrGlyAsnLeuProGlyCysSerPheSerIlePheLeuLeuAla  
GGCTTAACTATGCAACAGGGAACTTCTCTGTTGCTCTTCTCTATCTCTCTCTGCGCC  
CCGCACTTGATACGTTGTCCTTGGGAAGGACCAACGAGAAGAGATAGAAGCAAGACCGG

541 LeuLeuSerCysLeuThrValProAlaSerAlaTyrGlnValArgAsnSerThrGlyLeu  
CTGCTCTCTGCTTGAAGTGTGCGCGCTTCCGCTTACCAAGTCTGCAACTCCACGGGGCTT  
GACGAGAGAACGAAGTACACGGGGCAAGCTGATGTTTACGCTGAGGTGCTTGGCTGAA

601 TyrHisValThrAsnAspCysProAsnSerSerIleValTyrGluAlaAlaAspAlaIle  
TACCACGTACCAATGATGCGCCTAACTCGASTATTGTGTACGAGGCGCGGATGCCATC  
ATGCTGCAGTGGTTACTAACGGGATTGAGCTATAACACATGCTCCGCGGCTACGGTAG

Figure 1 (Sheet 1 of 10)

661 LeuHisThrProGlyCysValProCysValArgGluGlyAsnAlaSerArgLysTrpVal  
 CTGCACACTCCGGGGTGGCTCCCTTTCGTTCCGAGGGCAACGCCCTCGAGGTGTTGGGTG  
 GACGTGTGAGGGCCACGACAGGGAACGCAAGCACTCCCGTTGCGGAGCTCCACRACCCAC  
  
 721 AlaMetThrProThrValAlaThrArgAspGlyLysLeuProAlaThrGlnLeuArgArg  
 SCGATGACCCCTACGGTGGCCACCAGGATGSCAAACTCCCGCGACGCAGCTTCGACGT  
 CGCTACTGGGGATGCCACCGGTGGTCCCTACCGTTTGAGGGGGCGTGGCTGSAAGCTGCA  
  
 781 HisIleAspLeuLeuValGlySerAlaThrLeuCysSerAlaLeuTyrValGlyAspLeu  
 CACATCGAICTGCTTGTCGGGAGCGCCACCCCTCTGTTGCGCCCTCTACGTGGGGACCTA  
 GTGTAGCTAGACGAACAGCCCTCGCGGTGGGAGACAAGCCGGAGATGCACCCCTGGAT  
  
 841 CysGlySerValPheLeuValGlyGlnLeuPheThrPheSerProArgArgHisTrpThr  
 TCGGGCTCTGCTTCTTCTGTCGCCCAACTGTTCACCTTCTCTCCAGGGCGCCACTGGACG  
 ACGCCAGACAGAAAGAACAGCCGGTTGACAAGTGGAGAGAGGGTCCGCGGTGACCTGC  
  
 901 ThrGlnGlyCysAsnCysSerIleTyrProGlyHisIleThrGlyHisArgMetAlaTrp  
 ACGCAAGGTGCAATTCCTCTATCTATCCCGGCCATATAACGGGTCAACCGCATGGCATGG  
 TCGCTTCCAACGTTAACGAGATAGATAGGGCCGGTATATTGCCAGTGGCGTACCGTACC  
  
 Val  
 961 AspMetMetMetAsnTrpSerProThrThrAlaLeuValMetAlaGlnLeuLeuArgIle  
 SATATCATGATGAACCTGGTCCCTTACGACGGCGTTGGTAATGGCTCAGCTGCTCCGGATC  
 CTATACTACTACTTGACCAAGGGATGCTGCCGCAACCATTAACCGAGTCGACGAGGCCATG  
  
 1021 ProGlnAlaIleLeuAspMetIleAlaGlyAlaHisTrpGlyValLeuAlaGlyIleAla  
 CCACAAGCCATCTTGGACATGATCGCTGGTGTCACTGGGGAGTCTCCGGGGCATAGCG  
 GGTGTTCCGTAGAACCTGTACTAGCGACCACGAGTGACCCCTCAGGACCGCCCTGTATCGC  
  
 1081 TyrPheSerMetValGlyAsnTrpAlaLysValLeuValValLeuLeuPheAlaGly  
 TATTTCTCCATGGTGGGGAACTGGGCGAAGGTCTGGTAGTGCTGCTGCTATTTGCCGGC  
 ATAAAGAGGTACCAACCCCTTGACCCGCTTCCAGGACCATCAGGACGAGATAACCGGCC  
  
 1141 ValAspAlaGluThrHisValThrGlyGlySerAlaGlyHisThrValSerGlyPheVal  
 GTCCAGCGGAAACCCACGTACCGGGGAAGTGCCGGCCACACTGTGCTGGAATTGTT  
 CAGCTGCGCTTTGGGTGCAAGTGGCCCTTCCAGGCGCGGTGTGACACAGACCTAAACAA  
  
 1201 SerLeuLeuAlaProGlyAlaLysGlnAsnValGlnLeuIleAsnThrAsnGlySerTrp  
 AGCCTCCTCGCACCAGGCGCCAAGCAGAACGTCCAGCTGATCAACACCACGGCAGTTGG  
 TCGGAGGAGCGTGGTCCGCGGTTCGTCTTCAGGTTCGACTAGTTGTGGTTGCCGTCAACC  
  
 1261 HisLeuAsnSerThrAlaLeuAsnCysAsnAspSerLeuAsnThrGlyTrpLeuAlaGly  
 CACCTCAATAGCACGGCCCTGAACCTGCAATGATAGCCTCAACACCGGCTGGTTGGCAGGG  
 GTGGAGTTATCGTGCCGGGACTTGACGTTACTATCGGAGTTGTGGCCGACCAACCGTCCC  
  
 1321 LeuPheTyrHisHisLysPheAsnSerSerGlyCysProGluArgLeuAlaSerCysArg  
 CTTTCTATCACCACAAGTCAACTCTTCAGGCTGTCTGAGAGGCTAGCCAGCTGCCGA  
 GAAAGATAGTGGTGTTCAGTTGAGAAGTCCGACAGGACTCTCCGATCGGTCCAGCGCT  
  
 1381 ProLeuThrAspPheAspGlnGlyTrpGlyProIleSerTyrAlaAsnGlySerGlyPro  
 CCCCTTACCGATTTTGACCAAGGCTGGGGCCCTATCAGTTATGCCAACGGAAGCGGCCCC  
 GGGGAATGGCTAAACCTGGTCCCGACCCCGGATAGTCAATACGGTTGCCTTCGCCGGGG  
  
 1442 AspGlnArgProTyrCysTrpHisTyrProProLysProCysGlyIleValProAlaLys  
 GACCAGCGCCCTACTGCTGCACTACCCCAAAACCTTCCGGTATTTGCCCGCGAAG  
 CTGGTCCCGGGATGACGACCGTGATGGGGGTTTGGAAACGCCATAACACGGCGCTTC  
  
 1501 SerValCysGlyProValTyrCysPheThrProSerProValValGlyThrThrAsp  
 AGTGTGTGTTGGTCCGGIATATTGCTTCACTCCAGCCCGTGGTGGTGGGAACGACGAC  
 TCACACACACCGGCCATATAACGAAGTGAGGGTCCGGCCACCACCCCTTGTGGCTG  
  
 1561 ArgSerGlyAlaProThrTyrSerTrpGlyGluAsnAspThrAspValPheValLeuAsn  
 AGGTCCGGCGGCCACCTACAGCTGGCGTGAATATGATACGACGTCCTTCCTTAAC  
 TCCAGCCCGCGGGTGGATGTCGACCCCACTTTACTATGCTGCAGAGCAGGAATTG  
  
 AsnThrArgProProLeuGlyAsnTrpPheGlyCysThrTrpMetAsnSerThrGlyPhe

Figure 1 (Sheet 2 of 10)

1622 PATAACAGGCCACCGCTGGGCAATTGGTTCCGGTTGTACCTGGATGAACCTCAACTGGATTCT  
 TTATGGTCCGGTGGCGACCCGTTAACCAGGCAACATGGACCTACTTGAGTTGACCTAAG  
 ThrLysValCysGlyAlaProProCysValIleGlyGlyAlaGlyAsnAsnThrLeuHis  
 1691 ACCAAGTGTGCGGAGCGCCTCTTGTGTTCATCGGAGGGGCGGGCAACACACCCTGCAC  
 TGGTTTCACACGCTCGCGGAGGAACACAGTAGCCTCCCCGCCCGTTGTGTGGGACGTG  
 CysProThrAspCysPheArgLysHisProAspAlaThrTyrSerArgCysGlySerGly  
 1741 TGCCCCACTGATTGCTTCCGCAAGCATCCGGACGCCACATACTCTCGGTCCGGCTCCGGT  
 ACGGGGTGACTAACGAAGGCTTCCTAGGCCTGCGGTGTATGAGAGCCACGCCGAGGCCA  
 Ile  
 ProTrpLeuThrProArgCysLeuValAspTyrProTyrArgLeuTrpHisTyrProCys  
 1801 CCCGATCACACCCAGGTGCCCTGGTCCGACTACCCGTATAGGCTTTGGCATATCTTGT  
 GGGACCTAGTGTGGGTCCACGGACCACTGATGGGCATATCCGAAACCGTATAGGAACA  
 ThrIleAsnTyrThrIlePheLysIleArgMetTyrValGlyGlyValGluHisArgLeu  
 1861 ACCATCACTACACCATATTTAAATCAGGATGTACGTGGGAGGGGTGCAACACAGCTG  
 TGGTAGTTGATGTGGTATAAATTTTAGTCTACATGCCCTCCCCAGCTTGTGTCGAC  
 GluAlaAlaCysAsnTrpThrArgGlyGluArgCysAspLeuGluAspArgAspArgSer  
 1921 GAAGCTGCCTGCAACTGGACGCGGGGCGAACGTTGCCATCTGGAGACAGGGACAGGTCC  
 CTTGACGGACGTTACCTGCGCCCCGCTTGCAACGCTAGACCTTCTGTCTCTGTCCAGG  
 GluLeuSerProLeuLeuLeuThrThrThrGluTrpGlnValLeuProCysSerPheThr  
 1981 GAGCTCAGCCCGTTACTGTGACCACTACACAGTGGCAGGTCTCTCCGTGTTCTTCACA  
 CTCGAGTCGGGCAATGACGACTGGTGATGTGTACCGTCCAGGAGGGCACAAGGAAGTG  
 ThrLeuProAlaLeuSerThrGlyLeuIleHisLeuHisGlnAsnIleValAspValGln  
 2041 ACCCTACAGCCTTGTCCACCGGCTCATCCACCTCCACCAGAACAATTGTGGACGTGCAG  
 TGGGATGTTCCGAACAGGTGGCCGAGTAGGTGGAGGTGGTCTTGTAAACCTGCACGTC  
 TyrLeuTyrGlyValGlySerSerIleAlaSerTrpAlaIleLysTrpGluTyrValVal  
 2101 TACTTGTACGGGGTGGGGTCAAGCATCGCGTCTTGGGCCATTAAAGTGGGAGTACGTGCTT  
 ATGAACATGCCCCACCCAGTTCTGTAGCGCAGGACCCGGTAATTCACCTCATGCAGCAA  
 LeuLeuPheLeuLeuLeuAlaAspAlaArgValCysSerCysLeuTrpMetMetLeuLeu  
 2161 CTCTGTCTCTTCTGCTTGACAGACGCGCGCTGTGCTCTGCTTGTGGATGATGCTACTC  
 GAGGACAAGGAAGACCAACGCTCTCCGCGCGCAGACGAGGACGAACACCTACTACGATGAG  
 IleSerGlnAlaGluAlaAlaLeuGluAsnLeuValIleLeuAsnAlaAlaSerLeuAla  
 2221 ATATCCCAAGCGGAGGGCGCTTTGGAGAACCCTGTAATACCTTAATGCAGCATCTCTGGCC  
 TATAGGGTTCGCCTCCGCCGAAACCTCTTGGAGCATTATGAATTACGTCTGATGGGACCGG  
 GlyThrHisGlyLeuValSerPheLeuValPhePheCysPheAlaTrpTyrLeuLysGly  
 2281 GGGACGCACGGTCTTGTATCTCTCTCGTGTCTTCTGCTTTGCACTGATTTGAAGGGT  
 CCTGCGTGCCAGAACATAGGAAGGAGCACAGAAGACGAACGTTACCATAACTTCCCA  
 LysTrpValProGlyAlaValTyrThrPheTyrGlyMetTrpProLeuLeuLeuLeuLeu  
 2341 AAGTGGGTGCCCCGAGCGGTCTACACCTTCTACGGGATGTGGCCTCTCTCTGCTCTCTG  
 TTCACCCACGGGCTTCGCCAGATGTGGAAGATGCCCTACACCGGAGAGGAGGACGAGGAC  
 LeuAlaLeuProGlnArgAlaTyrAlaLeuAspThrGluValAlaAlaSerCysGlyGly  
 2401 TTGGCGTTGCCCGAGCGGCGTACGCGCTGGACACGGAGGTGGCCGCGCTCGTGTGGCGGT  
 AACCCCAACGGGGTCCGCCGATGCGCGACCTGTGCTCCACCGGCGACACACCGCCA  
 ValValLeuValGlyLeuMetAlaLeuThrLeuSerProTyrTyrLysArgTyrIleSer  
 2461 GTTGTCTCTGCGGGTGTGATGGCGCTGACTCTGTCACCATATTACAAGCGCTATATCAGC  
 CAACAAGAGCAGCCCACTACCGGACTGAGACAGTGGTATAATGTTCCGATATAGTCC  
 (Asn)  
 TrpCysLeuTrpTrpLeuGlnTyrPheLeuThrArgValGluAlaGlnLeuHisValTrp  
 2521 TGGTGCCTGTGGTGGCTTCACTATTTCTGACCAGAGTGGAGCGCACTGCACGTGTGG  
 ACCACGAACACACCGAAGTCATAAAGACTGGTCTCACCTTCGCGTTGACGTCCTACC  
 IleProProLeuAsnValArgGlyGlyArgAspAlaValIleLeuLeuMetCysAlaVal

Figure 1 (Sheet 3 of 10)

2581 ATTCCCCCCCCCAACGTCCGAGGGGGGGGGACGGCGTCATCTTACTCATGTGTGCTGTA  
 TAAGGGGGGGAGTTGCAGGCTCCCCCGCGCTGCGGCAGTAGAATGACTACACACCAT  
 HisProThrLeuValPheAspIleThrLysLeuLeuLeuAlaValPheGlyProLeuTrp  
 2641 CACCCGACTCTGGTATTTGACATCACCAAATTGCTGCTGGCGCTCTTCGGACCCCTTGG  
 GTGGCTGAGACATAAACTGTAGTGGTTTAACGACGACCGGCAGAGCCTGGGGAACC  
 IleLeuGlnAlaSerLeuLeuLysValProTyrPheValArgValGlnGlyLeuLeuArg  
 2701 ATTCTTCAAGCCAGTTTGCTTAAAGTACCCCTACTTTGTGGCGCTCCAGGCCCTTCTCCG  
 TAAGAAGTTCGGTCAAACGAATTCATGSGATGAACACCGCCAGGTTCCGGAAGAGGCC  
 PheCysAlaLeuAlaArgLysMetIleGlyGlyHisTyrValGlnMetValIleIleLys  
 2761 TTCTGCGCTTAGCGCGGAAGATGATCGGAGGCCATTACGTCAAATGGTCATCATTAAG  
 AAGACGCGCAATCGCGCCTTCTACTAGCCTCGGTAATGCAGCTTTACCATGTAATTC  
 LeuGlyAlaLeuThrGlyThrTyrValTyrAsnHisLeuThrProLeuArgAspTrpAla  
 2821 TTAGGGGGCGCTTACTGGCACCTATGTTTATAACCATCTCCTCTCTTCGGGACTGGGCG  
 AATCCCGCGAATGACTGTGGATACAAATATTGGTAGAGTAGGAGAAGCCCTGACCCGC  
 HisAsnGlyLeuArgAspLeuAlaValAlaValGluProValValPheSerGlnMetGlu  
 2881 CACAACGGCTTTCGAGATCTGGCCGTGGCTGTAGAGCCAGTCTCTCTTCCCAATGGAG  
 GGTGTCGCGAAGCTCTAGACCGGCACCGACATCTCGGTGAGCAGAGAGGGTTTACCTC  
 ThrLysLeuIleThrTrpGlyAlaAspThrAlaAlaCysGlyAspIleIleAsnGlyLeu  
 2941 ACCAAGCTCATCAGTGGGGGGCAGATACCGCCGCGTGGCGGTGACATCATCAACCCCTTG  
 TGGTTCGAGTAGTGACCCCCCGTCTATGGCGGCGCACGCCACTGTAGTAGTTGCCGAAC  
 ProValSerAlaArgArgGlyArgGluIleLeuLeuGlyProAlaAspGlyMetValSer  
 3001 CCTGTTTCGCGCCGCGAGGGCCGSGAGATACTGCTCGGGCCAGCCGATGGAATGGTCTCC  
 GSACAAAGCGCGCGCTCCCGGCCCTCTATGACGAGCCCGTGGCTACCTTACCAGAGG  
 LysGlyTrpArgLeuLeuAlaProIleThrAlaTyrAlaGlnGlnThrArgGlyLeuLeu  
 3061 AAGGGGTGGAGTTGCTGGCGCCATCACGGGTACGCCAGCAGACAAGGGGCTCCTA  
 TTCCCTACCTCCAAACGACCGCGGTAGTGCCGATGCGGTCGTCTGTCCCGGAGGAT  
 GlyCysIleIleThrSerLeuThrGlyArgAspLysAsnGlnValGluGlyGluValGln  
 3121 GGGTGATAATCACCAGCCTAACTGGCCGGGACAAAACCAAGTGGAGGGTGGAGTCCAG  
 CCCAGTATTAGTGGTGGATTGACCGGCCCTGTTTTTGGTTACCTCCCACTCCAGGTC  
 IleValSerThrAlaAlaGlnThrPheLeuAlaThrCysIleAsnGlyValCysTrpThr  
 3181 ATTGNTCAACTGCTGCCCAAACCTTCTGGCAACGTGCATCAATGGGGTGTCTGGACT  
 TAACAAGTIGACGACGGGTGTGAAGGACCGTTGCACGTAGTTACCCACACGACCTGA  
 ValTyrHisGlyAlaGlyThrArgThrIleAlaSerProLysGlyProValIleGlnMet  
 3241 GTCTACACGGGGCCGGAACGAGGACCATCGCGTCACCAAGGGTCTCTGTATCCAGATG  
 CAGATGCTCCCGGCCCTTGCTCTGGTAGCCAGTGGGTTCACGACAGTAGGTCTAC  
 TyrThrAsnValAspGlnAspLeuValGlyTrpProAlaProGlnGlySerArgSerLeu  
 3301 TATACCAATGTAGACCAAGACCTTGTTGGGCTGGCCCGCTCCGCAAGGTAGCCGCTCATTG  
 ATATGCTTACATCTGGTTCTGGAACACCCGACCGGGCGAGGCGTTCCATCGGCGAGTAAC  
 ThrProCysThrCysGlySerSerAspLeuTyrLeuValThrArgHisAlaAspValIle  
 3361 ACACCTTGCACTTGCGGCTCCTCGACCTTTACCTGGTCACGACGCACGCCGATGTCAAT  
 TGTGGGACGTGAACGCCGAGGAGCTTGGAAATGGACCAGTGCTCCGTGCGGTACAGTAA  
 ProValArgArgArgGlyAspSerArgGlySerLeuLeuSerProArgProIleSerTyr  
 3421 CCGGTGCGCCGGCGGGGTGATAGCAGGGGACGCTGCTGTGCGCCCGGCCCATTTCTTAC  
 GGGCAGCGCGCCGCCACTATCTCCCGTTCGAGACAGCGGGGCGGGTAAGGATG  
 LeuLysGlySerSerGlyGlyProLeuLeuCysProAlaGlyHisAlaValGlyIlePhe  
 3481 ITGAAAGGCTCCTCGGGGCTCCGCTGTTGTGCCCCCGGGGCCACGCCCTGGGCATATTT  
 AACTTTCGAGGAGCCCCCAGGCGACAACACGGGGCGCCCCGTGCGGCACCTGTATAAA  
 ArgAlaAlaValCysThrArgGlyValAlaLysAlaValAspPheIleProValGluAsn  
 3541 AGGGCCCGGCTGTGCACCCGTGGAGTGCTAAGGCGGTGGACTTTATCCCTGTGGAGAAC

Figure 1 (Sheet 4 of 10)

TCCCGSCGCCACACGTGGGCACCTCACCGATTCCGCCACCTGAAATAGGGACACCTCTTG  
 3601 LeuGluThrThrMetArgSerProValPheThrAspAsnSerSerProProValValPro  
 CTAGACACAACCATCAGGTCCCCSGTGTTCACGGATAACTCTCTCCACLAGTAGTGCCC  
 GATCTCTGTGTTGTAATCCAGGGGCCACAAGTGCCTATTGAGGAGAGGTGGTCATCACGGG  
 3661 GlnSerPheGlnValAlaHisLeuHisAlaProThrGlySerGlyLysSerThrLysVal  
 CAGAGTTTCCAGGTGGCTCACCTCCATGCTCCACAGGCAGCGGCAAAAGCACCAGGTC  
 GTCTGSAAGGTCCACCGAGTGGAGGTACGAGGGTGTCCGTGCGCGTTTTCGTGGTTCCAG  
 3721 ProAlaAlaTyrAlaAlaGlnGlyTyrLysValLeuValLeuAsnProSerValAlaAla  
 CCGGCTGCATATGCAGCTCAGGGCTATAAGGTGCTAGTACTCAACCCCTCTTTGCTGCA  
 GGCCGACGTATACGTGAGTCCCGATATTCACGATCATGAGTTGGGGAGACACGACGT  
 3781 Leu  
 ThrLeuGlyPheGlyAlaTyrMetSerLysAlaHisGlyIleAspProAsnIleArgThr  
 AACTTGGGCTTTGGTGGCTTACATGTCCAGGGTTCATGGGATCGATCTTAACATCAGGACC  
 TGTGACCCGAAACACGAAATGTACAGGTTCGAGTACCTAGCTAGGATTGTAGTCTCGG  
 3841 GlyValArgThrIleThrThrGlySerProIleThrTyrSerThrTyrGlyLysPheLeu  
 GGGGTGAGAACAAATTACCACTGGCAGCCCCATCACGTACTCCACCTACGGCAAGTTCTCT  
 CCCCCTCTCTGTTAATGGTGACCGTCCGGGTAGTGCATGAGGTGGATGCCGTTCAAGGAA  
 3901 AlaAspGlyGlyCysSerGlyGlyAlaTyrAspIleIleIleCysAspGluCysHisSer  
 GCGGACGGCGGGTCTCGGGGGCGCTTATGACATAATAATTTGTGACGAGTGGCACTCC  
 CGGCTSCCGCCACGAGCCCCCGCGAATCTGTATTATTAAACACTGCTCAGGTGAGG  
 (Val)  
 3961 ThrAspAlaThrSerIleLeuGlyIleGlyThrValLeuAspGlnAlaGluThrAlaGly  
 ACGGATGCCACATCCATCTTGGGCATCGGCCTGTCTTGACCAAGCAGAGACTGCGGGG  
 TGCTTACGGTGTAGGTAGAACCCGTAGCCGTGACAGGAAGTGGTTCGTCTTGACCCCC  
 4021 AlaArgLeuValValLeuAlaThrAlaThrProProGlySerValThrValProHisPro  
 GCGAGACTGGTGTGCTCGCCACCGCCACCCCTCGGGCTCCGTCACTGTGCCCCATCCC  
 CGCTCTGACCAACACGAGCGGTGGCGGTGGGGAGGCCGAGGCAGTGCACGGGTAGGG  
 4081 AsnIleGluGluValAlaLeuSerThrThrGlyGluIleProPheTyrGlyLysAlaIle  
 AACATCGAGGAGGTGTCTCTGTCACACCGGAGAGATCCCTTTTACGGCAAGGCTATC  
 TTGTAGCTCTTCAACGAGACAGGTGGTGGCCTCTCTAGGGAAAAATGCCGTTCCGATG  
 4141 ProLeuGluValIleLysGlyGlyArgHisLeuIlePheCysHisSerLysLysLysCys  
 CCCCTCGAAGTATCAAGGGGGGAGACATCTCATCTTCTGTCAATCAAGAAGAGTGC  
 GGGGAGCTTCAATTAGTTCCCGCCCTCTGTAGAGTAGAAGACAATAAGTTCTCTTCACG  
 4201 AspGluLeuAlaAlaLysLeuValAlaLeuGlyIleAsnAlaValAlaTyrTyrArgGly  
 GACGAACCTGCGGCAAGCTGTGCGCATTTGGCATCAATGCCGTGGCTACTACCGCGGT  
 CTGCTTGAGCGCGGTTTCCAGCAGCGTAACCGTAGTTACGGCACCGGATGATGGCGCCA  
 4261 LeuAspValSerValIleProThrSerGlyAspValValValAlaThrAspAlaLeu  
 CTGACGTGTCCGTCAATCCCGACCGGCGATGTTGTGCTCTGCGCAACCGATGCCCTC  
 GAACTGCACAGGCAGTAGGGCTGGTCTCCGCTACAACAGCAGCACCGTTGGCTACGGGAG  
 4321 Tyr  
 MetThrGlyTyrThrGlyAspPheAspSerValIleAspCysAsnThrCysValThrGln  
 ATGACCGGCTATACCGGGGACTTCGACTCGGTGATAGACTGCAATACGTGTGTACCCAG  
 TACTGGCCGATATGGCCGCTGAAGCTGAGCCACTATCTGACGTTATGCACACAGTGGGT  
 (Ser)  
 4381 ThrValAspPheSerLeuAspProThrPheThrIleGluThrIleThrLeuProGlnAsp  
 ACAGTCGATTTCAGCCTTGACCTACCTTACCATTGAGACAATCAGGCTCCCGCAGGAT  
 TGTGACCTAAAGTCGGAAGTGGGATGGAAGTGGTAACCTGTTAGTGGGAGGGGTCCTA  
 4441 AlaValSerArgThrGlnArgArgGlyArgThrGlyArgGlyLysProGlyIleTyrArg  
 GCTGTCTCCCGCACTCAACGTGGGGGAGGACTGGCAGGGGGAAGCCAGGCATCTTGA  
 CGACAGAGGGCGTGAGTTCCAGCCCCGTCTGACCGTCCCCCTTCGGTCCGTAGTCTCT

Figure 1 (Sheet 5 of 10)

CGGCGGGGGCCACGGCGATGACGGAAACACCGCGGACCGAATCGACCGGGCGGTAGCCG

552: SerValGlyLeuGlyLysValLeuIleAspIleLeuAlaGlyTyrGlyAlaGlyValAla  
AGTGTGGACTGGGGAAGGTCTCTCATAGACATCTTGCAGGATATGGCGGGGGCTGGCG  
TCACAACTGACCCCTTCCAGGAGTATCTGTAGGAACGTCCCATACCGGCCCCACCGC

(Gly)

5581 GlyAlaLeuValAlaPheLysIleMetSerGlyGluValProSerThrGluAspLeuVal  
GGAGCTCTTGTGGCATTCAAGATCATGACCGGTGAGGTCCCTCCACGGAGGACCTGGTC  
CCTCGAGAACACCGTAAGTCTTAGTACTCGCCTCCAGGGGAGGTGCCCTCTGGACCAG

5641 AsnLeuLeuProAlaIleLeuSerProGlyAlaLeuValValGlyValValCysAlaAla  
AATCTACTGCCGCCATCTCTCGCCGGAGCTCTGTAGTCTGGCGGTCTGTGTCAGC  
TTAGATGACGGGCGGTAGGAGAGCGGGCTCGGGAGCATCAGCCGACCAGACACGTCTG

5702 IleLeuArgArgHisValGlyProGlyGluGlyAlaValGlnTrpMetAsnArgLeuIle  
ATACTGCGCGGCGACGTCTGGCCCGGGCGAGGGGCGAGTGCAGTGCATGAACCGCTGATA  
TATGACCGGGCGGTGCAACCGGGCCCGCTCCCTCCGTACGTCACCTACTTGGCCGACTAT

5761 AlaPheAlaSerArgGlyAsnHisValSerProThrHisTyrValProGluSerAspAla  
GCCTTCGCCTCCCGGGGGAACCATGTTTCCCCACGCACTACGTGCCGGAGAGCGATGCA  
CGAAGCGGAGGGCCCCCTTGGTACAAAGGGGGTGCTGATGCACGGCCTCTCGCTACGT

(HisCys)

5821 AlaAlaArgValThrAlaIleLeuSerSerLeuThrValThrGlnLeuLeuArgArgLeu  
GCTGCCCGCGTCACTGCCATACTCAGCAGCCTCACTGTAAACCCAGCTCCTGAGGCGACTG  
CGACGGGCGCAGTGACGGTATGAGTCTGCGAGTGACATTGGGTGAGGACTCCGCTGAC

5881 HisGlnTrpIleSerSerGluCysThrThrProCysSerGlySerTrpLeuArgAspIle  
CACCAGTGCATAAGCTCGGAGTGTAACCACTCATGCTCCGGTTCTTGGCTAAGGCACATC  
GTGGTCACCTATTTCGAGCCTCACATGTTGAGGTACGAGGCCAAGGACCGATTCCCTGTAG

5941 TrpAspTrpIleCysGluValLeuSerAspPheLysThrTrpLeuLysAlaLysLeuMet  
TGGGACTGGATATGCGAGGTGTGAGCGACTTAAAGACCTGGCTAAAGCTAAGCTCATG  
ACCTGACCTATACGCTCCACAACCTCGCTGAAATTCGTGACCGATTTCGATTTCGAGTAC

6001 ProGlnLeuProGlyIleProPheValSerCysGlnArgGlyTyrLysGlyValTrpArg  
CCACAGCTGCTGGGATCCCCCTTGTGTCTGCTGCCAGCGCGGTATAAGGGGCTCTGCGGA  
GGTGTGACGGACCCCTAGGGGAAACACAGGACGGTTCGCGCCCATATTCCCCAGACCGCT

(Val)

6061 GlyAspGlyIleMetHisThrArgCysHisCysGlyAlaGluIleThrGlyHisValLys  
GTGGACGGCATCATGCACACTGCTGCCACTGTGGAGCTGACATCAGGACATGTCAAA  
CACCTGCCCTAGTACGTGTGAGCGACGGTGACACCTCGACTCTAGTGACCTGTACGTTT

6122 AsnGlyThrMetArgIleValGlyProArgThrCysArgAsnMetTrpSerGlyThrPhe  
ACGGGACGATGAGGATCTCGGTCTTAGGACCTGCAGGAACATGTGGAGTGGGACCTTC  
TTGCCCTGCTACTCTAGCAGCCAGGATCTTGGACGTCTGTACACCTCACCTTGGAAAG

6181 ProIleAsnAlaTyrThrThrGlyProCysThrProLeuProAlaProAsnTyrThrPhe  
CCCATTAATGCCCTACACCAGGGCCCCCTGTACCCCCCTTCTGCGCCGAACCTACAGTTC  
GGGTAATTACGGATGTGGTGCCCGGGGACATGGGGGAAGGACGCGGCTTGATGTGCAAG

6242 AlaLeuTrpArgValSerAlaGluGluTyrValGluIleArgGlnValGlyAspPheHis  
GCGCTATGCGGGTGTCTGCAGAGGAATATGTGGAGATAAGGCAGGTGGGGACTTCCAC  
CGGATACCTCCACAGACGTCTCTTATACACCTCTATTCCGTCCACCCCTGAAGGTG

6302 TyrValThrGlyMetThrThrAspAsnLeuLysCysProCysGlnValProSerProGlu  
TACGTGACGGGTATGACTACTGACAACTCTCAAATGCCCGTCCAGGTCCCATCGCCCGAA  
ATGCACCTGCCATACTGATGACTGTAGAGTTTACGGGACGGTCCAGGGTAGCGGGCTT

6361 PhePheThrGluLeuAspGlyValArgLeuHisArgPheAlaProProCysLysProLeu  
TTTTTCACAGAAATGACGGGGTGGCGCTACATAGGTTTGGCGCCCCCTGCAAGCCTTC  
AAAAAGTGTCTTAACCTGCCACGGCGATGTATCCAAACCGGGGGGACGTTTCGGGAAC

LeuArgGluGluValSerPheArgValGlyLeuHisGluTyrProValGlySerGlnLeu

Figure 1 (Sheet 7 of 10)

6421 CTGCGG3AGGAGGTATCATTAGAGTAGGACTCCAGAAIACCCGGTAGGGTCGCAATTA  
 GACGCCCTCCTCCATAGTAAGTCTCATCCTGAGGTCTTATGGCCATCCAGCGTTAAT  
 ProCysGluProGluProAspValAlaValLeuThrSerMetLeuThrAspProSerHis  
 6481 CCTTGGGAGCCCGAACCGGACGTGGCCGTGTTGACGTCCATGCTCACTGATCCCTCCCAT  
 GGAACGCTCGGGCTTGGCCTGCACCGGCACAACTGCAGGTACGAGTACTAGGGAGGGTA  
 IleThrAlaGluAlaAlaGlyArgArgLeuAlaArgGlySerProProSerValAlaSer  
 6541 ATAACAGCAGAGCGCGCGGCGGAAGGTTGGCGAGGGGATCACCCCCCTCTGTGGGCAGC  
 TATTGTCTCTCCGCCGCCCTTCCAACCGCTCCCTAGTGGGGGGAGACACCGGTGC  
 SerSerAlaSerGlnLeuSerAlaProSerLeuLysAlaThrCysThrAlaAsnHisAsp  
 6601 TCCTCGGCTAGCCAGCTATCCGTCCATCTCTCAAGGCAACTTGCACCGCTAACCTGAC  
 AGGAGCCGATCGGTTCATAGGCGAGGTAGAGAGTTCCGTGAACGTGGCGATTGTTACTG  
 SerProAspAlaGluLeuIleGluAlaAsnLeuLeuTrpArgGlnGluMetGlyGlyAsn  
 6661 TCCCCTGATGCTGAGCTCATAGAGCCCAACCTCCTATGGAGGCAGGAGATGGGCGCAAC  
 AGGGGACTACGACTCGAGTATCTCCGGTTGGAGGATACCTCCGTCTCTIACCGCGCTG  
 IleThrArgValGluSerGluAsnLysValValIleLeuAspSerPheAspProLeuVal  
 6721 ATCACCAGCGTTGAGTCAGAAACAAAGTGGTGATTCTGGACTCCTTCGATCCGCTTGTG  
 TAGTGGTCCCAACTCAGTCTTTTGTTCACCACTAAGACCTGAGGAAGCTAGGCGAACAC  
 AlaGluGluAspGluArgGluIleSerValProAlaGluIleLeuArgLysSerArgArg  
 6781 GCGGAGGAGGACGAGCGGGAGATCTCCGTACCCGAGAAATCTGCGGAAGTCTCGGAGA  
 CGCCTCCTCTGCTCGCCCTCTAGAGGCATGSGCGTCTTTAGGACGCTTCAGAGCCTCT  
 PheAlaGlnAlaLeuProValTrpAlaArgProAspTyrAsnProProLeuValGluThr  
 6841 TTGCCCCAGGCCCTGCCCCGTTTGGGCGCGGCCGACTATAACCCCCCGCTAGTGAGACG  
 AAGCGGTCCGGACGGGCAAAACCGCGCGGCTGATATTGGGGGGCGATCACCTCTGC  
 TrpLysLysProAspTyrGluProProValValHisGlyCysProLeuProProLys  
 6901 TGGAAAAAGCCCGACTACGACACCTGTGGTCCATGGCTGTCCGCTTCACCTCCAAAG  
 ACCTTTTTCGGGTGATGCTTGGTGGACACGAGTACCGACAGGCGAAGGTGGAGGTTTC  
 SerProProValProProProArgLysLysArgThrValValLeuThrGluSerThrLeu  
 6961 TCCCTCCTCTGCTCCGCTCCGACGAGCGGACGCTGCTCTCACTGAATCAACCTA  
 AGGGGAGGACACGAGGCGGACCTTCTTCCCTGCCACCGAGTGAAGTCTAGTTGGGAT  
 (Ser)  
 SerThrAlaLeuAlaGluLeuAlaThrArgSerPheGlySerSerSerThrSerGlyIle  
 7021 TCTACTGCCTTGGCCGAGCTCGCCACCAGAAGCTTTGGCAGCTCCTCAACTTCGGGATT  
 AGATGACGGAACCGGCTCGAGCGGTGGTCTTCGAAACGTCGAGGAGTTGAAGGCCATA  
 ThrGlyAspAsnThrThrThrSerSerGluProAlaProSerGlyCysProProAspSer  
 7081 ACGGSCGACAATACGACAACATCCTCTGAGCCCCCCCCCTTCTGGCTGCCCTCCCGACTCC  
 TCCCCGCTGTTATGCTGTTGTAGGAGACTCGGSCGGGGAAGACCGACGGGGGGCTGAGG  
 (PheAla)  
 AspAlaGluSerTyrSerSerMetProProLeuGluGlyGluProGlyAspProAspLeu  
 7141 GACGCTGAGTCTTATTCCTCCATGCCCCCCCTGGAGGGGGAGCTTGGGATCCGGATCTT  
 CTGCGACTCAGGATAAGGAGGTACGGGGGGGACCTCCCCCTCGGACCCCTAGGCCATGAA  
 SerAspGlySerTrpSerThrValSerSerGluAlaAsnAlaGluAspValValCysCys  
 7201 AGCGACGGGTCATGGTCAACGGTCACTAGTGAGGCCAACGCGGAGGATGTCGTGTCTGC  
 TCGCTGCCAGTACCAGTTGCCAGTCACTCTCCGCTTGGCGCTCTACAGCACACGACG  
 SerMetSerTyrSerTrpThrGlyAlaLeuValThrProCysAlaAlaGluGluGlnLys  
 7261 TCAATGCTTACTCTTGGACAGGCGCACTCGTCACCCCGTGGCGCGGGAAGAACAGAA  
 AGTTACAGAATGAGAACCCTGCCGCTGAGCAGTGGGACGCGGCGCCTTCTGTCTTT  
 LeuProIleAsnAlaLeuSerAsnSerLeuLeuArgHisHisAsnLeuValTyrSerThr  
 7321 CTGCCCATCAATGCACTAAGCAACTCGTTGCTACGTACCCACAATTGGTGTATTCCACC  
 GACGGGTAGTTACGTGATTCTGTGAGCAACGATGCAGTGGTGTAAACCATTAAGGTGG  
 ThrSerArgSerAlaCysGlnArgGlnLysLysValThrPheAspArgLeuGlnValLeu

Figure 1 (Sheet 8 of 10)



331 ACCTCAGCGAGTGTCTTGGCAAAGGCAGAAGAAAGTCACATTTGACAGACTGCAAGTTCTG  
 TCGAGTGCSTCACGAACGGTTTCCGTCTTCTTTCAGTGTAACCTGTCTGACGTTCAAGAC  
 AspSerHisTyrGlnAspValLeuLysGluValLysAlaAlaAlaSerLysValLysAla  
 441 GACAGCCATTACCAGGACGTACTCAAGGAGGTTAAAGCAGCGGCGTCAAAAGTGAAGGCT  
 CTGTCCGTAATGGTCTCTGCATGAGTTCTTCCATTTCGTCCCGCAGTTTTCACITCCGA  
 (Phe)  
 AsnLeuLeuSerValGluGluAlaCysSerLeuThrProProHisSerAlaLysSerLys  
 501 AACTTGCTATCCGTAGAGGAAGCTTGACGCTTGACGCCCCACACTCAGCCAAATCCAAG  
 TTGAACGATAGGCATCTCTTCCGAACGTCCGACTCGGGGGGTGTGAGTCCGTTTAGGTTT  
 PheGlyTyrGlyAlaLysAspValArgCysHisAlaArgLysAlaValThrHisIleAsn  
 561 TTTGGTTATGGGGCAAAGACGTCCGTTGCCATGCCAGAAAGGCCGTAAACCACATCAC  
 AAACCAATACCCGTTTCTGTCAGGCAACGGTACGCTCTTTCGGCATGGGGTGTAGTGTG  
 SerValTrpLysAspLeuLeuGluAspAsnValThrProIleAspThrThrIleMetAla  
 621 TCCGTCTGGAAGACCTTCTGGAAGACAATCTAACACCAATAGACACTACCATCATGGCT  
 AGGCACACCTTTCTGGAAGACCTTCTGTATACATTGTGGTTATCTGTGATGGTAGTACGA  
 LysAsnGluValPheCysValGlnProGluLysGlyGlyArgLysProAlaArgLeuIle  
 681 AAGAACGAGGTTTTCTGCGTTACGCTTGAGAAGGGGGGTCTGAAGCCAGCTCGTCTCATC  
 TTCTTGCTCCAAAAGACGCAAGTCCGACTCTTCCCCCAGCATTCCGTCGAGCAGAGTAG  
 ValPheProAspLeuGlyValArgValCysGluLysMetAlaLeuTyrAspValValThr  
 741 GTGTTCCCGATCTGGCGGTGCGGTGTGCGAAAAGATGGCTTTGTACGACGTGGTTACA  
 CACAAGGGGTAGACCCGCAACGCGCACAGCTTTCTACCGAAACATGCTGCACCAATGT  
 LysLeuProLeuAlaValMetGlySerSerTyrGlyPheGlnTyrSerProGlyGlnArg  
 7801 AAGCTCCCTTGGCCGTGATGSGAAGCTTCTACGGATTCCAATACTCACCAGGACAGCGG  
 TTCGAGGGGAACCGGCACTACCTTCGAGGATGCCTAAGGTTATGAGTGGTCTGTGCGC  
 ValGluPheLeuValGlnAlaTrpLysSerLysLysThrProMetGlyPheSerTyrAsp  
 7861 GTTGAATTCCTCGTGCAAGCGTGGAGTCCAAGAAAACCCCAATGGGGTTCTCGTATGAT  
 CAACTCAAGGAGCAGCTTCGCACCTTCAGGTTCTTTTGGGGTTACCCCAAGAGCATACTA  
 ThrArgCysPheAspSerThrValThrGluSerAspIleArgThrGluGluAlaIleTyr  
 7921 ACCCGCTGCTTTGACTCCACAGTCACTGAGAGCGACATCCGTACGGAGGAGGCAATCTAC  
 TGGGCGACGAAACTGAGGTGTCACTGACTCTCGCTGTAGGCATGCCTCTCTCGTTAGATG  
 GlnCysCysAspLeuAspProGlnAlaArgValAlaIleLysSerLeuThrGluArgLeu  
 7981 CAATGTTGTGACCTCGACCCCCAAGCCCGGTGGCCATCAAGTCCCTCACCAGAGGCTT  
 GTTACAACACTGGAGCTGGGGTTTCGGGCGCACCGGTAGTTTCAAGGAGTGGCTTCCGAA  
 (Gly)  
 TyrValGlyGlyProLeuThrAsnSerArgGlyGluAsnCysGlyTyrArgArgCysArg  
 8041 TATGTTGGGGGCCCTCTTACCAATTCAAGGGGGGAGAACTCCGCTATCCAGGTGCCGC  
 ATACAACCCCGGGAGAAATGGTTAAGTTCCCTCTCTTGACGCCGATAGCGTCCACGGCG  
 AlaSerGlyValLeuThrThrSerCysGlyAsnThrLeuThrCysTyrIleLysAlaArg  
 8101 GCGAGCGGCGTACTGACAACTAGCTGTGGTAACACCCCTCACTTGCTACATCAAGGCCCGG  
 CGCTCGCCGATGACTGTGATCGACACCATTTGTGGGAGTGAACGATGTAGTTCCGGGGC  
 AlaAlaCysArgAlaAlaGlyLeuGlnAspCysThrMetLeuValCysGlyAspAspLeu  
 9161 GCAGCCTGTGAGCCGCGAGGGCTCCAGGACTGCACCATGCTCGTGTGTGGCGACGACTTA  
 CGTCGGACAGCTCGGCGTCCCGAGGTCTGACGTGGTACGACACACACCCGCTGCTGAAT  
 ValValIleCysGluSerAlaGlyValGlnGluAspAlaAlaSerLeuArgAlaPheThr  
 9221 GTCGTTATCTGTAAAGCCGGGGGTTCAGGAGGACGCGCGAGCCTGAGAGCCTTCACG  
 CAGCAATAGACACTTTCGGCCCCCAGGTCTCTGCGCGCTCGGACTCTCGGAAGTGC  
 GluAlaMetThrArgTyrSerAlaProProGlyAspProProGlnProGluTyrAspLeu  
 9281 GAGGCTATGACCAGGTACTCCGCCCCCTGGGGACCCCCACAACCAATACGACTTG  
 CTCCGATACTGCTCATGAGGCGGGGGGACCCCTGGGGGGTGTGGTCTTATGCTGAAC  
 GluLeuIleThrSerCysSerSerAsnValSerValAlaHisAspGlyAlaGlyLysArg

Figure 1 (Sheet 9 of 10)

8341 GAGCTCATAACATCATGCTCCTCCAACGTTGTCAGTCGCCACGACGGCGCTGGAAAGAGG  
 CTCGAGTATTGTAGTACGAGGAGGTTGCACAGTCAGCGGGTGCTGCCGCGACCTTTCCTCC  
 ValTyrTyrLeuThrArgAspProThrThrProLeuAlaArgAlaAlaTrpGluThrAla  
 8401 GTCTACTACCTCACCCGTGACCCCTACACCCCTCGCGAGAGCTGCGTGGGAGACAGCA  
 CAGATGATGGAGTGGGCACTGGGATGTTGGGGGGAGCGCTCTCGACGCACCTCTGTCTGT  
 ArgHisThrProValAsnSerTrpLeuGlyAsnIleIleMetPheAlaProThrLeuTrp  
 8461 AGACACACTCCAGTCAATTCTGGCTAGGCAACATAATCATGTTTGGCCCCACACTGTGG  
 TCTGTGTGAGGTCAGTTAAGGACCGATCCGTCTATTAGTACAAACGGGGGTGTGACACC  
 AlaArgMetIleLeuMetThrHisPhePheSerValLeuIleAlaArgAspGlnLeuGlu  
 8521 GCGAGGATGATACTGATGACCCATTCTTTAGCGTCCTTATAGCCAGGGACCAGCTTGAA  
 CGCTCCTACTATGACTACTGGGTAAAGAAATCGCAGGAATATCGGTCCCTGGTCTGAACCT  
 GlnAlaLeuAspCysGluIleTyrGlyAlaCysTyrSerIleGluProLeuAspLeuPro  
 8581 CAGGCCCTCGATTGCGAGATCTACGGCGCCTGCTACTCCATAGAACCACCTTGATCTACCT  
 GTCCGGGAGCTAACGCTCTAGATGCCCGGACGATGAGGTATCTTGGTGAACCTAGATGGA  
 ProIleIleGlnArgLeuHisGlyLeuSerAlaPheSerLeuHisSerTyrSerProGly  
 8641 CCAATCATTCAAAGACTCCATGGCCTCAGCGCATTTTCACTCCACAGTTACTCTCCAGGT  
 GGTAGTAAGTTTCTGAGGTACCGGAGTCCGCTAAAGTGAGGTGCAATGAGAGGTCCA  
 GluIleAsnArgValAlaAlaCysLeuArgLysLeuGlyValPrcProLeuArgAlaTrp  
 8701 GAAATTAATAGGCTGGCCGCATGCCCTCAGAAACTTGGGGTACCGCCCTTGCGAGCTTGG  
 CTTTAATTATCCACCGGCGTACGGAGTCTTTGAACCCATGGCGGGAACGCTCGAACC  
 Gly  
 ArgHisArgAlaArgSerValArgAlaArgLeuLeuAlaArgGlyGlyArgAlaAlaIle  
 8761 AGACACCGGGCCCGGAGCGTCCGCGCTAGGCTTCTGGCCAGAGGAGGCGGGCTGCCATA  
 TCTGTGGCCCGGGCTCCGAGGCGGATCCGAAGACCGGTCTCTCCGTCCCGACGGTAT  
 CysGlyLysTyrLeuPheAsnTrpAlaValArgThrLysLeuLysLeuThrProIleAla  
 8821 TGTGGCAAGTACCTCTTCAACTGGGCAGTAAGAACAAAGCTCAACTCACTCCAATAGCG  
 ACACCGTTTATGGAGAAGTTGACCCGTCATTCTTGTTTCGAGTTTGAGTGAGGTTATCGC  
 AlaAlaGlyGlnLeuAspLeuSerGlyTrpPheThrAlaGlyTyrSerGlyGlyAspIle  
 8881 GCCGCTGGCCAGCTGGACTTGTCCGGCTGGTTACGGCTGGCTACAGCGCGGAGACATT  
 CGGCGACCGGTCGACCTGAACAGGCCGACCAAGTCCGACCGATGTCCGCCCTCTGTAA  
 (Pro)  
 TyrHisSerValSerHisAlaArgProArgTrpIleTrpPheCysLeuLeuLeuAla  
 9941 TATCACAGCGTGTCTCATGCCCGGCCCGCTGGATCTGGTTTTGCCTACTCTGTCTGTCT  
 ATAGTGTCCGACAGAGTACGGGCCGGGGCGACTAGACCAAAACGGATGAGGACGAACGA  
 AlaGlyValGlyIleTyrLeuLeuProAsnArgOP  
 9001 GCAGGGGTAGGCATCTACCTCTCCCAACCGATGAAGGTTGGGGTAAACACTCCGGCCCT  
 CGTCCCATCCGTAGATGGAGGAGGGGTTGGCTACTTCCAACCCCATTTGTGAGGCCGGA

Figure 1

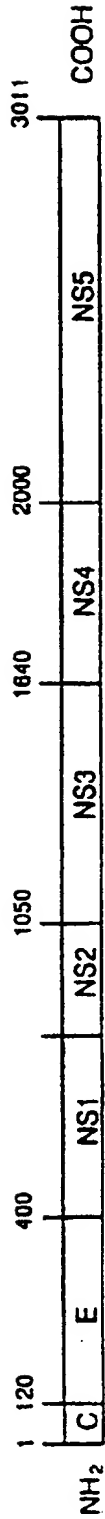


Figure 2



European Patent  
Office

# EUROPEAN SEARCH REPORT

Application Number

EP 91 30 2910

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 5)
Y	WO-A-8 904 669 (CHIRON CORP.) * Page 39, lines 8-12; page 49, line 5 - page 50, line 31; page 123, line 29 - page 125, line 22; page 132, line 3 - page 134, line 35; page 171, lines 4-20 *	1-16	G 01 N 33/576 C 07 K 15/00
Y,D	EP-A-0 318 216 (CHIRON CORP.) * Page 15, line 39 - page 17, line 8; page 18, line 44 - page 19, line 13; page 27, lines 10-22 *	1-16	
A	SCIENCE, vol. 244, 21st April 1989, pages 362-364, Washington, DC, US; G. KUD et al.: "An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis" * Whole article *	1-16	
			TECHNICAL FIELDS SEARCHED (Int. Cl. 5)
			G 01 N C 07 K
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 02-07-1991	Examiner VAN BOHEMEN C.G.
<p><b>CATEGORY OF CITED DOCUMENTS</b></p> <p>X: particularly relevant if taken alone Y: particularly relevant if combined with another document of the same category A: technological background O: non-written disclosure P: intermediate document</p> <p>T: theory or principle underlying the invention E: earlier patent document, but published on, or after the filing date D: document cited in the application L: document cited for other reasons</p> <p>*: member of the same patent family, corresponding document</p>			

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